Novel Analytical Techniques for Studying the Milk Fat Globule Membrane

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Jacobus Meindert Evers

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Abstract

Fat in milk and cream is present as tiny droplets, which are each enveloped in a thin membrane, called the milk fat globule membrane (MFGM). The MFGM can easily be damaged by factors such as pumping the milk and applying other forms of agitation. MFGM damage is believed to reduce processing efficiency and compromise the quality of manufactured products.

A comprehensive review of the literature showed that our understanding of changes occurring in the MFGM post secretion of the fat globule by the mammary secretory cell is still rudimentary. Furthermore, it was found that a fundamental understanding of MFGM damage in raw milk is lacking. Hence, this study sought to develop analytical techniques for studying the MFGM.

Fluorescent probes were identified that associated with the MFGM (bovine, ovine, human) in one of two ways: either by embedding in the phospholipid bilayer (lipophilic probe) or by binding to carbohydrate moieties of glycosylated chains in the glycocalyx (lectin probes). The use of these probes, in combination with either conventional fluorescence microscopy or confocal laser scanning microscopy, allowed 2-D images and 3-D images of fat globules to be made. Application of water-soluble lipophilic probes and the lectin wheat germ agglutinin (WGA) directly to milk allowed the staining of the MFGM in its native environment. Variable distribution patterns of the probes in the MFGM were observed, which suggests that the MFGM of fat globules in harvested milk is structurally and chemically heterogeneous both within and among globules from the same species and between species, and even among fat globules within the milk of an individual animal. Furthermore, the binding behaviour of WGA to the MFGM of native fat globules (in bovine milk) and washed fat globules (in model systems) following heat treatment implicated β-lactoglobulin, α-lactalbumin, immunoglobulin M and/or the glycosylated proteins Periodic acid Schiff 6/7 in the disappearance of fat globule aggregation upon elevated heat treatment of milk. The results of the current study showed that the use of membrane-specific fluorescent probes, particularly in combination with confocal laser scanning microscopy, has significant potential for providing real time structural and chemical information about the MFGM in matrices such as harvested milk and milk products.

In addition to the fluorescence microscopy techniques, development of other techniques was also conducted. Flow cytometry was shown to have significant potential for the quantitative determination of various properties of fat globules and their membranes. Although no suitable sample preparation technique could be developed in this study, atomic force microscopy is believed to have significant potential for studying structural and physical properties of the MFGM. Selective harvesting of individual fat globules was shown to be possible by using a micromanipulator. In future work, this technique is expected to be used in combination with fluorescence microscopy, or atomic force microscopy.

The present study has shown that the development and application of novel analytical techniques has advanced, and in the future will further advance, understanding of the MFGM.

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The great Creator of the universe of which I was privileged to study a tiny, tiny part. Delving into the microscopic world I found this to be true, "For the invisible things of him from the creation of the world are clearly seen, being understood by the things that are made, even his eternal power and Godhead" (Romans 1:20). Marvellous are thy works; and that my soul knoweth right well (Psalm 139:14b).

Take my life, and let it be
consecrated, Lord, to thee;
take my intellect, and use
every power as thou shalt choose.[1]

[1] Abridged from Frances Ridley Havergal (1874).

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15um Tip 2 Glob 1 harvest.wmv .... On accompanying CD

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ShM 3% 6 WGA cav 63x zoom6_chan00.avi .... On accompanying CD

List of abbreviations

5,5'-Ph_2-$DiIC_{18}$(3)	1,1'-Dioctadecyl-5,5'diphenyl-3,3,3',3'-tetramethylindocarbocyanine chloride
AFM	Atomic force microscope/microscopy (depending on context)
BSA	Bovine serum albumin
BTN	Butyrophilin
CD36	Cluster of differentiation 36
CLSM	Confocal laser scanning microscope/microscopy (depending on context)
ConA	Concanavalin A
DiA	4-(4-(Dihexadecylamino)styryl)-*N*-methylpyridinium iodide
$DiIC_{18}$(3)	1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate
$DiIC_{18}$(3)-DS	1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-5,5'-disulfonic acid
$DiOC_{18}$(3)	3,3'-Dioctadecyloxacarbocyanine perchlorate
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
D109	5-Dodecanoylaminofluorescein
FFA(s)	Free fatty acid(s)
FFMR	Free frozen milk fat for recombining
FITC	Fluorescein isothiocyanate
FM1-43	*N*-(3-Triethylammoniumpropyl)-4-(4-(dibutylaminostyryl)pyridinium dibromide
FM1-84	*N*-(3-Triethylammoniumpropyl)-4-(4-(dipentylaminostyryl)pyridinium dibromide
FM4-64	*N*-(3-Triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)-pyridinium dibromide
GlcNac	*N*-Acetyl-D-glucosamine
GMP	Glycomacropeptide
Ig(G, M)	Immunoglobulin (G, M)
MDCK cells	Madin-Darby canine kidney cells
MFGM	Milk fat globule membrane
MUC1	Mucin 1
NANA	*N*-Acetylneuraminic acid
NMR	Nuclear magnetic resonance
N316	6-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoic acid
PAS 6/7	Periodic acid Schiff 6/7
PBS	Phosphate buffered saline
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
SP- $DiIC_{18}$(3)	1,1'-Dioctadecyl-6,6'-di(4-sulfophenyl)-3,3,3',3'-tetramethylindocarbocyanine
SP- $DiOC_{18}$(3)	3,3'-Dioctadecyl-5,5'-di(4-sulfophenyl)-oxacarbocyanine, sodium salt
TEM	Transmission electron microscopy
UHT	Ultra-high temperature
WGA	Wheat germ agglutinin
WPI	Whey protein isolate
XO	Xanthine oxidase