both lateral tension and bending rigidity of the nanotube membrane can be extracted. The obtained results are in good agreement with the data reported by different techniques for similar lipid compositions. Hence the electric field can be utilized for measurement of mechanical parameters of tubular membrane, specifically, short and/or narrow tubules which are not readily accessible by conventional techniques.

1807-Pos Board B651

Surface Behaviour of Peptoid Mimics of Pulmonary Surfactant Protein SP-C: Captive Bubble Surfactometry

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Pulmonary surfactant lipopeptide SP-C modulates the surface properties of interfacial films required to stabilize the respiratory interface along breathing dynamics. Several attempts have been made to produce entirely synthetic analogs of SP-C suitable to develop potentially useful therapeutic preparations.

In this study we have tested the potential of five different poly-*N*-substituted glycines, or peptoids, designed to mimic (roughly) the primary and secondary structure and hydrophobicity of SP-C, to produce acceptable surfactant-like behaviour once incorporated into lipid/peptoid suspensions and assessed in a captive bubble surfactometer (CBS). The surface activities of different peptoids were compared in two model lipid mixtures: DPPC/POPG/Palmitic acid (68/22/9), which resembles the lipid composition of several clinical surfactants currently in use, and DPPC/POPC/POPG/Chol (50/25/15/10), which mimics the balance of saturated/unsaturated and zwitterionic/anionic phospholipids and the cholesterol content of natural surfactant as purified from bronchoalveolar lavage. We have assessed the ability of the different lipid/peptoid suspensions to i) rapidly adsorb at the bubble air-liquid interface, ii) stably produce very low surface tensions upon relatively slow repetitive quasi-static compressions and iii) maintain the lowest surface tensions with minimal compression and hysteresis under rapid physiological-like compression-expansion dynamics. Significant differences were found between different peptoids differing in their backbone structure and hydrophobicity, with some of the peptoids mimicking efficiently the effect of native SP-C, usually at larger proportions of peptoids than required for the natural protein.

1808-Pos Board B652

Looking at Lipid Domains in Stratum Corneum Lipid Models using Vibrational Microspectroscopy

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The impermeability of the skin is intimately related to the structure of the stratum corneum (SC), the top layer of the epidermis. The large fraction of SC lipids existing in a solid/crystalline form is believed to be a key factor in the low permeability of the skin barrier. We have characterized, using Raman and infrared microspectroscopies, the mixing properties of model mixtures that included ceramide, free fatty acids, and sterol, the 3 main lipid components of SC. We show that, in ternary mixtures with palmitic acid and cholesterol, the transformation of sphingomyeline, a precursor of ceramide, into ceramide leads to an increase of the heterogeneity of the spatial lipid distribution, in parallel with an increase of the chain order. Therefore the enzymatic conversion of sphingomyeline in ceramide leads to the transformation of a homogeneous and relatively disordered matrix into a heterogeneous matrix containing crystalline domains. This heterogeneity in lipid composition was observed from the microscopic local variations of the relative areas of the C-H stretching and the C-D stretching bands, the fatty acids being deuterated in our model mixtures. The thermal evolution of the mixing properties of the ceramide/palmitic acid/cholesterol mixtures indicated that an increase of temperature (above 50 °C) leads to the disordering of the fatty acid and, to a lesser extent, of ceramide. In parallel to this melting, a mixing of the lipid species is observed as the areas enriched in palmitic acid were also enriched in cholesterol. These results suggest the formation of a liquid ordered phase mainly composed of palmitic acid and cholesterol; this phase may ensure the cohesion between the solid domains. The recording of spectra from several microscopic voxels provides a unique description of the phase composition of these model mixtures.

1809-Pos Board B653

Comparative Studies On Bovine And Rat Pulmonary Surfactants Using AFM

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Recent studies employing a variety of microscopic techniques have revealed that phospholipid (PL) phase separations and transitions may play important roles in determining the biophysical properties of pulmonary surfactant. Most of these microscopic studies used model systems which were composed of simple mixtures of PL with or without hydrophobic surfactant proteins and cholesterol. The present work compared modified natural (lipid extract) bovine and rat surfactants using atomic force microscopy (AFM). AFM revealed PL phase separation upon compression of both surfactant monolayers, and a monolayer-to-multilayer transition at surface pressure 40-50 mN/m. Similar to bovine surfactant, the tilted-condensed (TC) phase in rat surfactant consisted of domains both on micrometer and nanometer scales. Upon film compression, the microdomains were dissociated into nanodomains, thus forming a more homogeneous two-phase mixture. Differences between rat and bovine surfactants were: (1) more TC domains were formed at lower surface pressures in rat than in bovine surfactant; and (2) an interesting domain-in-domain structure was exclusively observed in rat surfactant. These structural differences were attributed to the higher cholesterol content of rat surfactant (~ 10 vs ~2.5 wt%). To further investigate the effects of cholesterol on the structure of surfactant films, we have studied cholesterol-depleted bovine surfactant (~ 0%) prepared by repetitive acetone extraction. Removal of cholesterol from bovine surfactant induced significant variations in film structure. More importantly, the film structure can be effectively restored by recombining cholesterol with the cholesterol-depleted bovine surfactant. Recombinant bovine surfactant with 10% cholesterol showed domain-in-domain structures similar to those found with rat surfactant. These interspecies studies of the micro- and nano- structures of natural pulmonary surfactants add insight into the biophysical interpretation of phospholipid phase transition and separation, in particular the role of cholesterol.

1810-Pos Board B654

Differences in Lateral Membrane Organization in Fibroblasts Expressing Low and High Levels of the Influenza Viral Protein Hemagglutinin

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Lateral organization in cell membranes is crucial for biological processes such as endocytosis, signaling, protein transport, membrane trafficking and viral infection. Hemagglutinin (HA) is an influenza viral envelope transmembrane protein which has been shown to be associated with the liquid ordered (l_o) phase. Fibroblasts that constitutively express HA, referred to as HAb2 cells, were used to characterize the lateral organization of HA. Since HAb2 cells have cell-to-cell variability in the membrane density of HA, cells with low and high expression levels of HA containing more consistent densities of HA were also used. Fluorescence correlation spectroscopy (FCS), confocal microscopy and fluorescence photoactivation localization microscopy (FPALM) were used to characterize membrane organization after labeling cells with fluorescent probes and/or transfecting with either EGFP-HA or Dendra2-HA. Preliminary FCS results show that the diffusion of the liquid-disordered (l_d) phase probe Lissamine Rhodamine DOPE is similar in cells with high and low HA expression levels i.e. the amount of HA present does not influence the diffusion time. Confocal microscopy was used to study the effect of HA expression level on the extent of phase separation observed after blebbing was induced by DMSO treatment. FPALM was used to obtain details about membrane organization at the nanometer length scale.

1811-Pos Board B655

Determination Of The Lipid Membrane Composition Of J774 Macrophages Cells Surexpressing Mrp Protein (resistant To Ciprofloxacin)

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Ciprofloxacin (CIP) is a fluoroquinolone antibiotic with an activity towards both extracellular and intracellular bacteria (Seral *et al.*, 2005). Diffusion and efflux processes modulate accumulation of this drug within eukaryotic cells. When J774 macrophages were grown in presence of ciprofloxacin, the antibiotic is subject to constitutive efflux through the activity of an MRP-related transporter (Michot *et al.*, 2004).

In view of the critical role of lipids for both drug uptake and activity of MRP proteins (Hinrichs et al, 2004), together with the ability of fluoroquinolones to interact with lipids (Bensikaddour *et al.*, 2008 (a,b)), we investigated the composition of lipids in resistant and sensitive J 774 macrophages to ciprofloxacin. Firstly, we characterized by thin layer chromatography the phospholipids composition of J774 macrophages cells sensitive (WT) and resistant to ciprofloxacin (CIP). Results showed that sphingomyelin (SM) decreased 2 times whereas phosphatidylinositol increased 1.5 fold in resistant cells. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and cholesterol didn't show any significant change. Secondly, we studied membrane fluidity of liposomes

mimicking WT and CIP resistant J774 cells, by fluorescence polarization spectroscopy. In this respect, we observed a decrease in the melting temperature in vesicles mimicking the membrane composition of CIP resistant cells, indicating that changes in the fluidity of these membranes may be due to the decrease of SM. Studies are currently performed to investigate potential changes in other components of rafts like glucosylceramides. These data might have important relevance to relate the interaction of fluoroquinolones with lipids and change in the processes involved in cellular accumulation of fluoroquinolones.

1812-Pos Board B656

The Effect of Mepivacaine·HCl on the Physical Properties of Liposomes of Total Lipid and Phospholipids Extracted from Neuronal Membranes

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The aim of this study was to provide the basis to further examine the mode of action of local anesthetic. Fluorescent probe techniques were used to evaluate the effect of mepivacaine·HCl on the physical properties (transbilayer asymmetric lateral and rotational mobilities, membrane thickness) of liposomes of total lipid (SPMVTL) and phospholipids (SPMVPL) extracted synaptosomal plasma membrane vesicles (SPMV) isolated from bovine cerebral cortex. An experimental procedure was used based on selective quenching of 1,3-di(1-pyrenyl)propane (Py-3-Py) and 1,6-diphenyl-1,3,5-hexatriene (DPH) by trinitrophenyl groups. Membrane thickness was measured by using energy transfer between the surface fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS) and the hydrophobic fluorescent probe Py-3-Py. Mepivacaine·HCl increased the bulk lateral and rotational mobilities, and had a greater fluidizing effect on the inner monolayer than outer monolayer of liposome. The thickness of SPMVTL, SPMVPL lipid bilayer have been decreased by mepivacaine·HCl, which means that the membranes have been expanded. It is judged that the region for the decreased thickness of SPMVTL, SPMVPL lipid bilayer by mepivacaine·HCl is due to the lateral and rotational mobility of the SPMVTL, SPMVPL lipid bilayer was found to be increased by mepivacaine·HCl. The magnitude of increasing effect of mepivacaine·HCl on lateral and rotational mobilities of both SPMVTL, SPMVPL lipid bilayer was significantly far smaller than the magnitude of those of SPMV lipid bilayer. The sensitivities to the increasing effect of the lateral and rotational mobilities of the liposomal lipid bilayer by the local anesthetic differed depending on the native and model membranes in the descending order of SPMV, SPMVPL and SPMVTL. These effects are not only due to the influence of the local anesthetic on lipids, but they are magnified by the interaction between lipids, proteins and water.

1813-Pos Board B657

Analysis of Lipid Exchange in Patients with Urinogenital Fistula

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Affection of urinary and sexual systems in women occurs with cell membranes trauma. Therefore origin and shaping fistula connected directly or indirectly with lipid exchange breach in patient's organism. Hence study lipid exchange in patients with the fistula of urinogenital systems in women is actual.

Our data showed that the phospholipids level was significantly lower (7.82%) in the experimental group (patients) than that of control group. The level of triglycerides in the experimental group was about 3 fold higher than in the control (healthy) group. Importantly, the level of cholesterol in the patients was substantially higher (62.0%) than that of control group.

From final products of parameters of peroxidation process malondialdehyde level in blood 13.2% higher than in control, diene conjugates in patients nearly 24.8% higher norm. Average molecular mass reliably higher than in checking group, but antioxidizing activity level decreased 10 % from the norm.

Change of lipids group composition and their sex affects to ferments activity. For example, in patients catalase activity in blood serum (7.28 ± 0.68 mmol/(min*ml)) almost by 2.58 % higher than in healthy people (5.80 ± 0.63), but superoxidismutase activity by 40 % higher than the norm.

Individual composition of fatty acids in examined patients' blood was studied and established that content of palmitic C(16:0), palmitoleic C(18:0) and arachidone C(20:4) in contrast with checking, by 11.4%, 34.5% and 65.4% increased accordingly. Contents of other acids were realistically reduced. From findings it is possible to establish that content of phospholipids with the parameter of lipids peroxidation and ferments activity parameters have the following correlation: with malondialdehyde (0,320), diene conjugate (0,266), catalase (0,216) and superoxidismutase (0,198) have a weak correlation, but with antioxidizing activity (0,480) and average molecular mass (0,4) average correlation. Phospholipid content with C(18:3) has a high correlation (0,721).

1814-Pos Board B658

Cell Membrane Electrical And Order Properties Under Microwaves Irradiation

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The degree of packing and mobility of phospholipidic molecules within a biological membrane are crucial for the latter to accomplish its physiological functions (e.g. transport across membrane, signaling processes etc). Consequently, any physical and/or chemical factor which affects the membrane order, may affect also its function.

We studied the effect of 2.45 GHz microwaves irradiation on both biological and model membranes (dimiristoyl phosphatidyl choline liposomes), monitoring the following parameters:

- membrane fluidity, by fluorescence depolarization of TMA-DPH,
- membrane generalized polarization, by modifications of emission spectra of Laurdan,
- membrane potential, by fluorescence quenching of $DiSC_3(5)$.

The irradiation was performed directly in the spectrofluorometer, with a specially designed antenna and the temperature was continuously monitored using an optical fiber thermometer. Membrane fluidity, generalized polarization and potential were measured continuously during the irradiation.

In parallel experiments the same thermal evolution of the system as in the case of microwave irradiation was simulated by means of a computer controlled Peltier thermostat.

The dependency of the monitored parameters on the temperature in both cases (MW irradiation and ''thermal'' heating) was analyzed.

In the case of liposomes we observed a rising of the transition temperature by a few degrees centigrade, depending on the applied microwaves power.

The results are interpreted in terms of membrane destabilization by water penetration in the lipidic bilayer above the critical temperature, which seems to be affected by the presence of the electromagnetic field. The effects are very clear in the case of model membrane, but less evident and much more complex in the case of living cells.

1815-Pos Board B659

Amifostine, a Radioprotectant Agent, Protects Rat Hepatic Microsomal Membranes Against Ionizing Radiation Induced Damage

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In the present study, the protective effect of amifostine (WR-2721), which is the only approved radioprotective agent by the Food and Drug Administration (FDA), was investigated against the deleterious effects of ionizing radiation on rat liver microsomal membranes at molecular level. To achieve this, Sprague-Dawley rats, which were administered amifostine or not, were whole-body irradiated using Cobalt-60 irradiator at a single dose of 800 cGy, decapitated after 24 h and the microsomal membranes isolated from the livers of these rats were analyzed using FTIR spectroscopy. The results revealed that ionizing radiation caused a significant increase in the concentration of lipids whereas amifostine treatment restored the lipid content of microsomal membranes to control values. In addition, the significant increase in lipid order and a significant decrease in membrane dynamics resulting from ionizing radiation were prevented by amifostine. While ionizing radiation caused a significant decrease in the lipid to protein ratio, amifostine injection before radiation, maintained this ratio as in the control group. Furthermore, ionizing radiation-induced variations in protein secondary structure were restored by amifostine. In conclusion, the data obtained in this study indicate that amifostine administration to the rats prior to whole body irradiation protects liver microsomal membranes against the radiation induced damages. Supported by TUBITAK, (SBAG-2939) and by the METU (BAP-2006-07-02-0001).