2P281**Investigation of pH-induced membrane-fusion properties of baculovirus by microscopic observation of individual giant unilamellar vesicles**

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Fusion of viral envelopes and biomembranes is a critical step in infection of an envelope virus such as Sendai virus, influenza virus, etc. In the early stage of the infection, the envelope glycoproteins interact with receptors on cell surfaces, leading to the fusion directly on the plasma or endosomal membranes. In the case of baculovirus that infects insect cells, the budded virus particles (BVs) have the fusogenic envelope glycoprotein gp64, which is activated at the low pH inside endosomes. In past studies, it has been shown that gp64 interacts with acidic phospholipids on the membranes during the fusion phenomena; however, the details of effects of membrane properties (phospholipid contents, fluidities, etc.) on fusion of baculovirus and cell membranes seem unclear. Therefore, in this work, we try investigating these fusion properties of baculovirus by confocal microscopic observation on individual giant unilamellar vesicles (GUVs) mixed with BVs labeled with Alexa Fluor 488. We could make sure that the intensity of the fluorescence emitted from GUV bilayers reflected the efficiency of viral fusion: The fusion curve with GUVs and that obtained by fluorometry on conventional liposomes are similar. Using this assay, we showed that the fusion of a GUV and BVs was drastically promoted at pH 5.5, which is near the endosomal pH, that GUVs including DOPS or DOPA underwent more efficient fusion than those including DOPG, and that the fluidity modulated by addition of cholesterol to GUVs affected the fusion efficiency reasonably.

2P283**Study on the Lipid Domain of Mammalian Stratum Corneum by Electron Diffraction.**○Akira Yamagishi¹, Isao Takagishi¹, Tomohiro Imai¹, Hiromitsu Nakazawa¹, Shingo Sakai², Shintaro Inoue², Satoru Kato¹¹School of Science&Technology, Kwanseigakuin Univ., ²Kanebo Cosmetics Inc.

As the outermost layer of the mammalian skin, the stratum corneum (SC) lipid is elaborately designed to protect the body from the harmful environment such as dryness and exposure to toxic agents. Recent x-ray diffraction studies have suggested that the lateral packing structure of the intercellular lipid, especially orthorhombic packing structure, plays a critical role in the formation and maintenance of the barrier properties of the skin. There are some reports that the lattice defects between adjacent packing domains have a serious effect on the barrier function. Here we employed a noninvasive electron diffraction method to analyze the local packing structure of the SC intercellular lipid instead of x-ray diffraction which requires a greater amount of SC sample and gives information only on the average structure. We used a copper electron microscope grid coated by glue to collect a film of corneocytes. The orthorhombic packing structures often gave a diffraction pattern with localized reflection peaks, indicating anisotropic distribution while the hexagonal packing structures tended to give a Debye-Scherrer ring. These results suggest that in human SC there exist a relatively large region with orthorhombic packing and randomly distributed small domains with hexagonal packing. We could analyze the micro-domain structure of SC lipid using electron diffraction. We now evaluate the distribution of lipid packing domains obtained from electron diffraction pattern in detail.

2P282**Study on the Packing Structure of the Stratum Corneum Lipid Model by Electron Diffraction**○Isao Takagishi¹, Akira Yamagishi¹, Hiromitsu Nakazawa¹, Shingo Sakai², Shintaro Inoue², Satoru Kato¹¹Dept. of Science and Technology, Kwansei-Gakuin Univ., ²Kanebo Cosmetic Inc.

One of the important roles of the stratum corneum (SC), the outermost layer of the skin, is a barrier against transepidermal water loss and penetration of harmful agents. The SC consists of keratinized corneocytes surrounded by intercellular lipids, which are mainly composed of ceramide, cholesterol and free fatty acid. The lateral packing structures of intercellular lipids are thought to be a key factor for the barrier properties of the skin. The electron diffraction (ED) method is useful for analysis of the local packing structures in a noninvasive manner though the strong interaction between the electron beam and the sample causes rapid decay of diffraction intensity. In this study we tried to establish the optimal experimental conditions for ED observation of intact SC samples. For this purpose we examined the influence of electron beam damage using a SC lipid model, which has the lipid organization similar to that observed in native SC. The artificial SC lipid model was composed of ceramide, cholesterol and free fatty acid. Without a cryostage which has been employed to reduce the beam damage, the combination of a detector with high sensitivity and an appropriate electron dose made it possible to result in a clear electron diffraction pattern from highly ordered lipids (hexagonal and orthorhombic packing structures). We propose unimproved method for the electron diffraction analysis of the SC structure.

2P284**Effect of cations on stratum corneum lipid monolayer**○Takashi Kojima, Masanao Kinoshita, Hiromitsu Nakazawa, Satoru Kato
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The uppermost layer of the skin, the stratum corneum (SC), consists of keratinized corneocytes surrounded by intercellular lipid layer which contain ceramide, cholesterol and free fatty acid. Water-soluble substances such as cations, hyaluronic acid and other natural moisturizing factors are also present in the extracellular region (lipid region) of SC. So far as we know, there is no information about the physicochemical interaction of water-soluble substances with intercellular lipids. Here we examined the interaction between cations and the lipid components of SC by surface pressure-area isotherm measurements. Monolayers of various combinations of ceramide, cholesterol and palmitic acid were prepared with or without cations in the subphase. The average molecular area in a ceramide/cholesterol/palmitic acid mixture was increased by addition of KCl into the subphase. In contrast the isotherms of pure palmitic acid showed the reduction of molecular area under the same conditions probably because of electronic screening effect. In the case of pure cholesterol KCl had almost no effect on the isotherm. Since the pure ceramide monolayer was affected by KCl in the same way as the ternary mixture, ceramide may play a key role in the interaction between lipids and KCl in the SC model membrane.