



## Detection of highly abundant small molecules in the stratum corneum of healthy young women using desorption electrospray ionization-mass spectrometry imaging

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## 論文題目

Detection of highly abundant small molecules in the stratum corneum of healthy young women using desorption electrospray ionization-mass spectrometry imaging  
(脱離エレクトロスプレーイオン化質量分析イメージングによる健康な若年女性の皮膚角層に特徴的な低分子の検出)

## 論文の内容の要旨

### [Introduction]

Skin is a major indicator of aging, and aging considerably affects skin appearance, diminishing skin juvenescence. The appearance of the skin is closely related to the stratum corneum, the skin's outer layer. Until present, limited investigations on the molecules in the stratum corneum underlying skin juvenescence have been conducted. In this regard, we sought to investigate possible compounds in the stratum corneum that may be responsible for skin juvenescence.

Several techniques have recently been used to analyze skin conditions by imaging the skin depending on its physical attributes. Aside from this, mass spectrometry-based investigations are more sophisticated since they provide molecular information at the same time. Mass spectrometry imaging (MSI) analysis, one of the mass spectrometric methods, offers a new dimension to the bio-molecular distribution analysis in the skin. Among all MSI, desorption electrospray ionization-mass spectrometry imaging (DESI-MSI) is a rapid in-situ molecular imaging technique. For straightforwardness and rapidness, in this study, we utilized DESI-MSI for detecting compounds exclusively distributed in the stratum corneum layers of the young-aged female skin.

### [Materials and Methods]

The study was approved by the ethics committee of the Hamamatsu University School of Medicine, Hamamatsu, Japan (ethical approval number: 19-123). Eleven healthy women between the ages of 24 and 59 were recruited and separated into two groups: young (24 to 31 years old) and middle-aged (41 to 59 years old). Before collecting stratum corneum samples, each subject's face was cleansed and allowed to dry in a temperature and humidity-controlled facility. Stratum corneum samples were obtained from the cheek four times by 1 cm<sup>2</sup> tape-stripping using cellophane tape.

Following that, samples of tape stripped stratum corneum were adhered to a glass slide and analyzed using the DESI-MSI method. The DESI-MSI ion image was investigated using HDImaging software (Version 1.4; Waters, Milford, MA, USA). Regions of interest (ROIs) were manually drawn on each sample area to calculate average intensity

by HDImaging software. Average signal intensities of individual pixels of the ROIs were then compared between groups.

#### [Results]

We investigated the DESI ion distribution of tape-stripped stratum corneum samples in positive ion mode. We examined the most prominent 300 DESI-MSI peaks ranging from  $m/z$  100 to  $m/z$  1000 and discovered five molecules ( $m/z$  284.33,  $m/z$  340.39,  $m/z$  488.39,  $m/z$  628.37, and  $m/z$  863.65) are highly abundant in the stratum corneum of the young-aged subject compared to the middle-aged subjects. Among them  $m/z$  284.33 was 122.03%,  $m/z$  340.39 was 196.74%,  $m/z$  488.39 was 345.69%,  $m/z$  628.37 was 265.49%, and  $m/z$  863.65 was 137.06% highly abundant in average intensity in young aged subjects compared to middle-aged subjects.  $m/z$  284.33 and  $m/z$  340.39 were notably observed among all detected candidate molecules in each stratum corneum layers of all young subjects. Interestingly, the most noticeable distribution of  $m/z$  488.39 and  $m/z$  628.27 was identified in subject number 4 from the young aged panel. Furthermore,  $m/z$  284.33,  $m/z$  340.39,  $m/z$  488.39,  $m/z$  628.37,  $m/z$  863.65 were most abundant in the first stripped stratum corneum of the young subject 3 and 4 and gradually decreased in the subsequent strippings.

#### [Discussion]

DESI-MSI was used successfully in our investigation to detect abundant molecules in the stratum corneum of young healthy women. DESI-MSI is a label-free and quick in-situ molecular imaging technology that can visualize the distribution of smaller biomolecules in organic samples. Among the candidate molecules,  $m/z$  284.33 and  $m/z$  340.39 prominently exist in all stratum corneum of all young subjects. Our finding surmises that these molecules might be highly related to skin juvenescence. To determine the association between these molecules and young-aged skin, molecular identification and further investigation are essential. Interestingly subject 4 of the young-aged subjects showed the most prominent distribution of  $m/z$  488.39, and  $m/z$  628.27 in it's stratum corneum. This most remarkable abundance emphasizes the unique characteristics of this young individual, who has very smooth skin with too little pigmentation. In future it would be beneficial to deepen research to find out exact relationship between different skin parameters with the retention capacity of these candidate molecules. Although with the current findings, it is not clear that the detected candidate molecules were endogenous or retained from cosmetics applied on the skin, in the future, it is indispensable to identify and characterize them to consider them for inclusion in product development for promoting skin juvenescence.

[Conclusion]

Five molecules ( $m/z$  284.33,  $m/z$  340.39,  $m/z$  488.39,  $m/z$  628.37, and  $m/z$  863.65) are highly abundant in young-aged subjects, which might be co-related with skin juvenescence.