

Non-invasive analysis to monitor skin aging conditions: screening of chemical modifications on keratin proteins by mass spectrometry

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Purpose: Human skin is one of the major barriers of our body against environmental stimuli such as UV and chemical exposures. Therefore, as the main constituent of the skin, keratins have recently been recognized as the major target proteins to various chemical modifications. However, because of the difficulties associated with their insolubility and handling, there have been lack of studies to identify the sites of chemical modification in keratins. Here, we introduce a combination of non-invasive sampling, simple clean-up, efficient digestion method, and MS analysis to screen keratin modifications. This approach would provide significant information of the chemical modifications including oxidative modifications on keratins that could be useful as biomarkers of the skin damage.

Experimental: The analytical methodology to detect chemical modifications on skin keratins were optimized using tape stripping, filter-aided sample preparation, tryptic digestion, MALDI-TOF/MS *etc.* The tryptic keratins in human skin with/without H₂O₂ treatment were analyzed by MALDI-TOF/MS to confirm the oxidative modification sites.

Results and Discussion: We have developed the methodology to identify chemical modifications in human skin keratins. Using the methodology, we have identified that Met^{259, 262, 295, 469}, located in the α -helical rod domain of K1, were the most susceptible sites to oxidation induced by H₂O₂ in vitro and in vivo.