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Electron Microscopy and Reflectance Confocal Microscopy Reveal Altered Epidermal Differentiation following Collagen Glycations

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Glycation of proteins such as collagen impairs their functionality and triggers pro-inflammatory pathways as well as increases oxidative stress within the tissue [1]. Since advanced glycation end products (AGEs) accumulate with donor age, glycation matters not only in diabetes patients, but also presents a hallmark of intrinsic skin aging. Previous studies found decreased filaggrin and loricrin expression, suggesting an impaired skin barrier function.

Herein, we studied the effect of collagen glycation on the epidermal morphology and the barrier function of reconstructed human skin (RHS). Normal human dermal fibroblasts and keratinocytes were isolated from juvenile foreskin. Collagen was pre-incubated with ribose to induce AGE products formation. Fluorescence spectroscopy confirmed collagen glycation. The effects of glycated collagen were compared to RHS with non-glycated collagen. Glycated and non-glycated RHS were subjected either to electron microscopy or to reflectance confocal microscopy.

Reflectance confocal microscopy swiftly analyzes RHS morphology without damaging the constructs. In accordance with in vivo data, collagen appeared more lumpy in glycated than in non-glycated RHS. Moreover, reflectance confocal microscopy revealed increased epidermal thickness of glycated RHS. Electron microscopy confirmed a thicker stratum corneum with plenty of cell layers of stratum granulosum at the expense of the stratum spinosum of glycated RHS. Moreover, the sharp distinction between stratum corneum and granulosum vanishes. Keratohyalin granula appear fine-grained and distributed throughout the keratinoytes within glycated RHS compared to non-glycated RHS, where the cells reveal more aggregated granules. The altered morphology of keratohyalin granula points to differences in keratinocyte differentiation [2]. No differences were observed in keratinocytes in the stratum basale: both glycated and non-glycated RHS are closely attached to the dermal compartment by an intact

basal lamina. Differences in collagen structure become also visible by electron microscopy. Caffeine permeation decreases by about 15% compared to non-glycated RHS, being well in accordance to in vivo studies with intrinsically-aged volunteers [3].

In conclusion, both electron microscopy and reflectance confocal microscopy prove a thicker stratum corneum with hallmarks of altered epidermal differentiation of reconstructed human skin with glycated collagen. Further studies will focus on the molecular cross-talk between glycated collagen and epidermal differentiation.

References

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