für Angewandte Wissenschaften

Life Sciences und Facility Management

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Förderagentur für Innovation KTI

innovating for your success

mibelle biochemistry

Tissue Engineering for Drug Development

Defined Organotypic Skin Models

Diego Santini[1], Epifania Bono[1], Daniel Schmid[2], Stephanie Mathes[1], Ursula Graf-Hausner[1]

1 Institute of Chemistry and Biological Chemistry, Zurich University of Applied Sciences, Wädenswil, Switzerland

2 Mibelle Biochemistry, Buchs, Switzerland

Introduction

Diabetis is a widespread disease and numbers of patients are supposed to rise to 380 million within the next 20 years. Therefore products, which are able to minimize secondary effects of diabetis, will be of demanding interest. As the blood sugar level in diabetic patients is increased, expanded occurance of advanced glycated end (AGE) products can be observed. These products are generated when sugars interact with lysine residues of amino acids in a non-enzymantic reaction and result in a crosslinking of proteins. In skin collagen I is the most prominent matrix protein. AGE formation in collagen structures result in a decreased matrix elasticity.

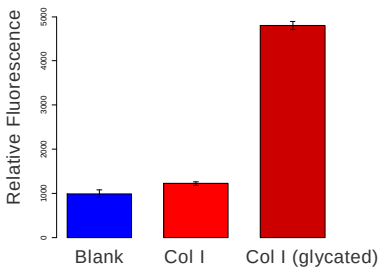
Project aim

The aim of the project presented here was to establish and characterize a three dimensional skin model, which exhibits a glycated dermal compartment, similar to diabetic skin.
Such a specialized model is intended to serve as a tool to study the cellular pathways of skin cell answers as well as the impact of potent drugs.

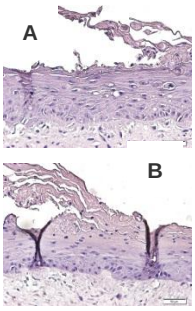
Materials and Methods

For collagen gylcation a collagen solution (5mg/ml in 0.02N acetic acid) was mixed with methylglyoxal (MGO), in order to obtain a final collagen concentration of 3mg/ml in acetic acid 0.5N enriched with MGO (final concentration: 250mM). The mixture was incubated for 64h at 22°C. After dialysis and measurement of AGE product formation (λem 440nm and λex 355nm) the solution was mixed with the same volume of non-glycated collagen solution and human primary fibroblasts (Provitro, Germany) to a final concentration of $1.22 \cdot 10^4$ cells/ml. The cellular hydrogels were incubated until contraction in DMEM/F12 medium. Human primary keratinocytes (Provitro, Germany) were seeded on top of the gels and cultivated submerged for 2-4 days before exposing the cells to the air interface. Models were harvested after 10-11 days after the air-lift and analysed by histological and immunhistological methods.

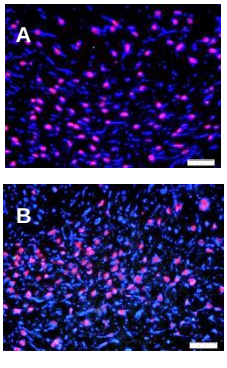
Results and Discussion



Glycation of Collagen I was effective, as shown by increased fluorescence intensities (n=3) and delayed gel contraction (data not shown) by fibroblasts. As the underlying Maillard reaction is hardly controllable, a batch-to batch variation occurred, resulting in different fluorescence intensities. Nevertheless, the glycated matrix was always polymerizable and feasible for organotypic skin model creation.



As for non-glycyted collagen (A) all epidermal layers were established on a gylcated dermal matrix (B) and epidermal thickness was about the same size as on native collagen matrix. But there was a difference in epidermal layer morphology. Keratinocytes seemed to differentiate in lower epidermal layers when cultivated on a gylcated collagen matrix.



Immunhistological analysis of vimentin (blue) and nuclei counterstain with propidium iodite (red) revealed an interesting phenomenom. The distribution of intermediary filaments of fibroblasts differd in glycated (B) collagen I hydrogels from the unglycated control (A). Fragmentation of vimentin was obviously increased. The fact, that vimentin plays a major role in AGE related skin alteration, was already shown in 2D fibrobalsts cell cultures by Kueper et al (Ann. N.Y. Acad. Sci., 2008).

Conclusion

In order to create organotypic skin models, which simulate the characteristics of aged or diabetic skin, the following conclusions can be drawn:

- Glycation of collagen I by a non-enzymatic Maillard reaction is hardly controllable
- Epidermal differentiation is altered on glycated dermal equivalent
- Vimentin distribution is altered in glycated 3D dermal models

We gratefully acknowledge the financial support of CTI Project No. 8626.2 PFLS-LS