Plastic compression for generating optimized in vitro skin equivalents

Brendtke R [1,2] | Rütschle I [3] | Beck C [1] | Schwarz T [1,3] | Wiehl M [2] | Walles H [1,3] | Hansmann J [1,3]

[1] Tissue Engineering and Regenerative Medicine | University Hospital Würzburg | Würzburg | Germany

[2] senetics healthcare group GmbH & Co.KG | Erlangen | Germany

[3] Translational Center Wuerzburg ´Regenerative therapies in oncology and musculoskeletal diseases` Wuerzburg branch of the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB) | Wuerzburg | Germany

Objective

In vitro skin equivalents are proven to be useful as both skin grafts and *in vitro* test systems. However, a major drawback during cultivation of the skin equivalents is a contraction up to 70%. In clinical application this results in extensive scarring. Further, the insufficient stability of the test systems limits cultivation to just a few days. Thus, the investigation of many biological processes that occur over a period of weeks is not possible. Hence, we established plastic compression of the dermal equivalents to a defined dimension in order to reduce contraction and set up model systems with a higher reproducibility level.

Materials & Methods

We investigated the mechanical properties of a dermal equivalent after plastic compression with linear motion steering. During this process, collagen hydrogels are compressed to an increasing concentration with a defined force, thereby enhancing both the mechanical stability of the hydrogel and the parallel arrangement of the collagen filaments. We developed a system which allows a defined compression and the mechanical characterization of collagen hydrogels.

Results

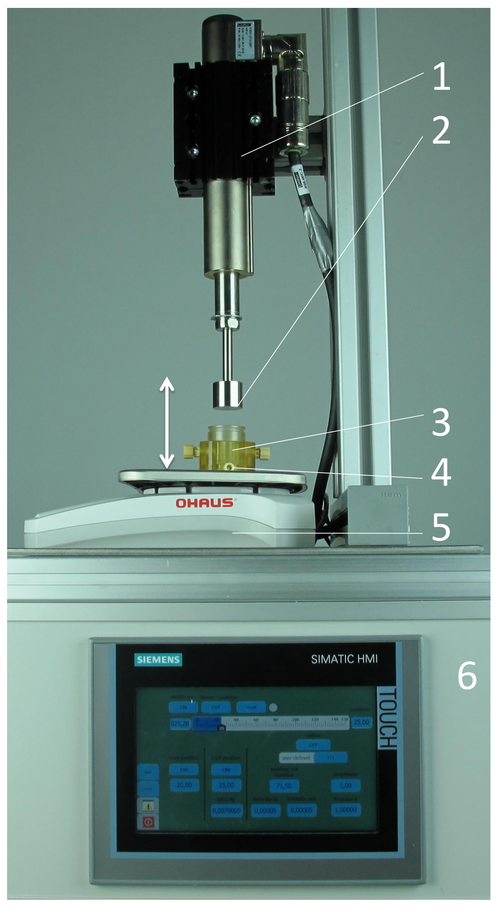

Figure 1: Plastic compression system allowing generation of collagen hydrogels exhibiting adjustable collagen concentrations. During hydrogel processing, hydrogels are compressed to a defined volume of V=3.6 cm² employing a linear motion engine (1). The squeezer (2) compresses the hydrogel (3) occupying the bioreactor. Surplus water was drained via the access near the bottom of the chamber (4). The scale allows force measurements (5)

Using the plastic compression system (Fig. 1) we obtained assimilation towards the mechanical properties of *ex vivo* dermis samples (Fig. 2a) together with an absence of self-contraction (Fig. 2c,d). Moreover, we were able to generate models with a topographical surface structure using the plastic compression system in order to mimic the human papillary structure (results not shown). After compression, the survival of primary fibroblasts, seeded into the collagen hydrogel, was identified by MTT and live dead staining (Fig. 2b).

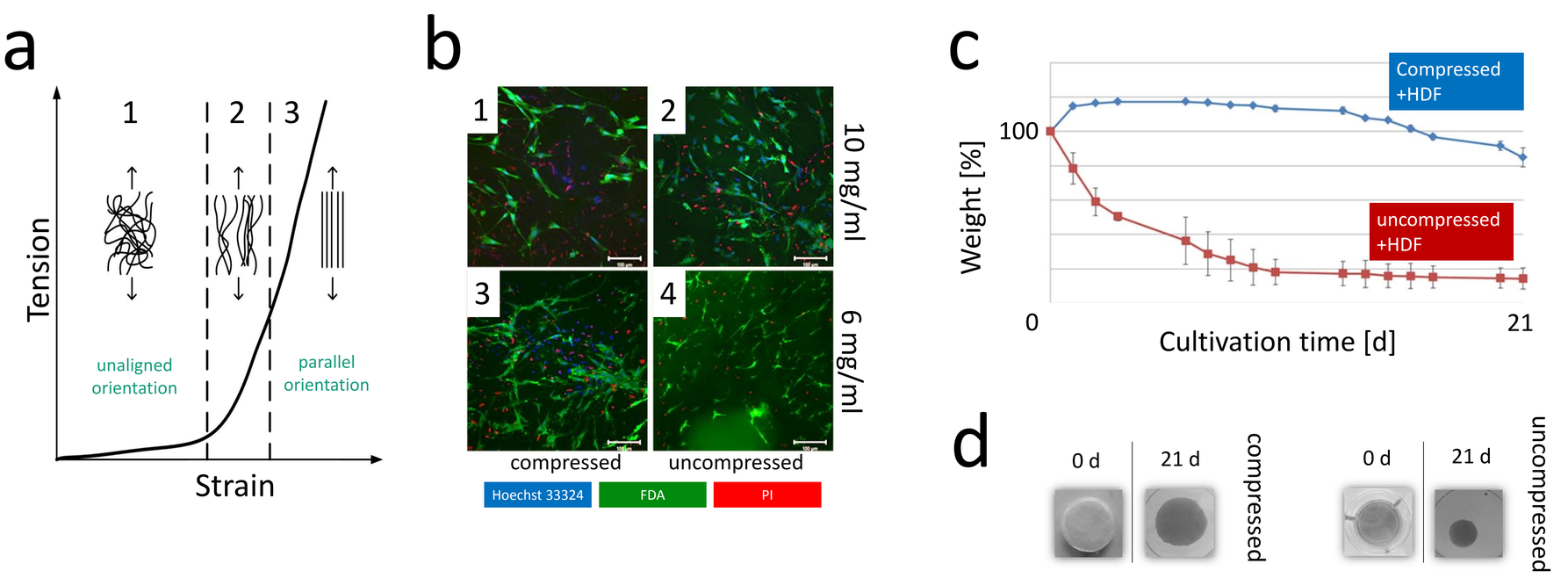

Figure 2: (a) Tension– strain– diagram of the dermal fiber network during force transmission, adapted by [1]. By carrying out pressure onto the uncompressed hydrogels, the unaligned collagen fibers (1) get a higher degree of parallel orientation (2). This leads in fact to a direct action of the forces from the squeezer onto the collagen fibers that get orientated completely parallel (3). **(b)** Three dimensional LSM images displaying collagen hydrogels containing human dermal fibroblasts (HDF) at a depth of 60 - 100 µm after 17 days of cultivation. A life- dead staining was performed using Hoechst 33342 (blue) for nuclei, fluorescein diacetate (FDA, green) for metabolic active cells und propidium iodide (PI, red) for dead cells. The LSM images display compressed (1, 3) and not compressed (2, 4) collagen hydrogels that were cultivated with HDF. Images 1 and 2 show hydrogels concentrated at c=10mg/ml, images 3 and 4 display hydrogels with a lower concentration of c=6mg/ml. All scale bars indicate 100 µm. **(c)** Weight progression of compressed and not compressed collagen hydrogels containing HDF at a concentration of c=6mg/ml during a cultivation period of 21 days. Uncompressed hydrogels show a shrinking of appr. 70 % compared to initial state. The recorded curves display the mean and standard deviation of three individual samples. **(d)** Images of the shrinking rate with and without compression of the collagen hydrogels with HDF at a concentration of c=6mg/ml. Not compressed gels show a clear shrinking of appr. 70% compared to compressed gels that only display a minor shrinking rate.

Summary

The established bioreactor system was proven to be capable of both compression and culturing *in vitro* skin equivalents. Compression nearly cuts down the shrinking process of the models thereby enabling a good attachment of the epidermal layer as there is no shriveling of the dermis. This is especially useful when looking at the production of 3d skin models with an epidermal layer. We also showed that the plastic compression process does not harm the viability of the human dermal fibroblasts. In both processes, more orientated collagen fibers and a structured surface of the models are able to mimic the *in vivo* situation even better in order to perform testing closely related to the human skin and its compartments.

Outlook

In our study we established a bioreactor for optimizing both compression and culturing *in vitro* skin equivalents. The system can, in perspective, be used for cultivation of test systems under perfusion to mimic the *in vivo* situation with stable nutrition supply.

References

H. J. Levis, R. a Brown, and J. T. Daniels, “Plastic compressed collagen as a biomimetic substrate for human limbal epithelial cell culture.,” Biomaterials, vol. 31, no. 30, pp. 7726–37, Oct. 2010

C. H. Daly, “Biomechanical properties of dermis.,” The Journal of investigative dermatology, vol. 79 Suppl 1, p. 17s–20s, Jul. 1982

Contact

Rico Brendtke
senetics healthcare group GmbH & Co.KG | Henkestraße 91 | 91052 Erlangen | Germany
phone +49 9131 9 201 201 | fax +49 9131 9 201202 | rico.brendtke@senetics.de