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The Open Source Concept – Shaping the future of animal-free tests

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Introduction

Henkel has developed a human reconstructed epidermal model based on a protocol originally published by *Poumay* et al. (2004). With this tissue equivalent a test to predict the skin-irritating potential of chemicals was established. In a multi-laboratory study the test system was validated with a set of reference chemicals according to the ECVAM Performance Standards for *in vitro* skin irritation testings (catch-up validation study). The study data were submitted to the ECVAM to be reviewed by the respective expert groups, a prerequisite for the official regulatory acceptance of the test.

The OS-REp model

The epidermal equivalent is propagated as the **Open Source Reconstructed Epidermis (OS-REp).** This means that

- protocols and quality criteria for tissue production and irritation testings will be published in peer-reviewed journals after official regulatory acceptance;
- production and application of the test system is completely free of any commercial or legal restrictions.

For the OS-REp model production quality criteria have been defined which must be met mandatory at each production site:

- Histological architecture similar to native human skin
- Viability of untreated epidermal equivalents (MTT assay: 0.8 > OD_{NC} <1.5)
- Barrier function ($ET_{50} \geq 3$ hours)

OD_{NC}: optical density of negative control
ET_{50}: effective time at 50% viability after topical treatment with TRITON X-100

The Tissue Factory

In order to gain regulatory acceptance not only for the skin irritation test system, but also for the OS-REp model itself, the successful transferability of the production protocols to other production sites has to be be demonstrated, which is currently realized within the framework of a cooperation between the Henkel AG & Co. KGaA and the Fraunhofer Gesellschaft. The Fraunhofer Gesellschaft has started to adopt the Henkel protocol for the OS-REp production, aiming at producing the tissue equivalents fully automated with their proprietary **Tissue Factory**.

The automated manufacturing of the OS-REp model is executed in three process steps reflecting the architecture of the Tissue Factory (Fig. 2).

Specifications:

- Fully-automated high-scale manufacture of OS-REp tissues
- High quality and reproducibility at low production costs
- 100% quality control via non-invasive methods, e.g. Optical Coherence Tomography (OCT)

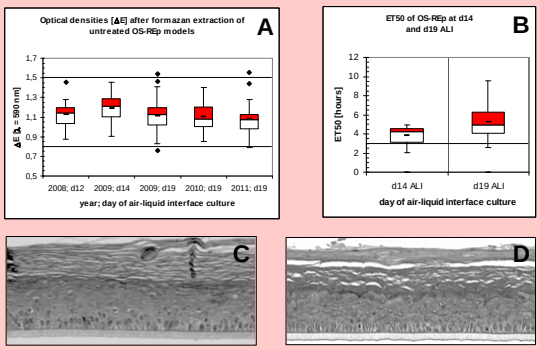

Fig. 1: (A) Overview of **OS-REp viability** from batches produced at the Henkel laboratory. While the culture time was successively extended, mean tissue viability remained constant throughout 4 years of production. (B) **Barrier function**, determined as the ET_{50} after topical TRITON X-100 treatment. **Histological sections** through OS-REp models produced manually at Henkel (C) or automatically at Fraunhofer (D). All layers of native human skin, including a multi-layered *Stratum corneum*, are well developed, **the overall tissue architecture is identical**.

Fig. 3: (A) **Cell Extraction** of primary keratinocytes from human biopsies; (B) **Cell Expansion** of primary keratinocytes in monolayer culture; (C) **Tissue Cultivation:** build-up of the 3D OS-REp model

Conclusion

The Open Source Concept is intended to pave the way towards an unrestricted access to *in vitro* test methods in future. In particular this concept is currently realized with the OS-REp epidermal equivalent as an alternative for skin irritation testings, to our knowledge for the first time in the framework of alternative methods. It will be extended to other alternative methods, e.g. a human corneal equivalent for eye irritation testings in the near future.