Impedance Spectroscopy for the Characterization of reconstructed human epidermis and the Assessment of Skin Toxicity

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INTRODUCTION

Despite advances in the development of in-vitro-tissue-models such as reconstructed human epidermis (RHE), the number of endpoints in toxicity-testing, which can be addressed with these models is limited[1]. Besides other limitations this is due to a dependency on invasive methods such as histological processing or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining. To overcome this pitfall, we developed a non-destructive technology to analyze the integrity of the epidermal barrier based on impedance spectroscopy (ImpSpec) as an alternative for destructive methods[2].

MATERIALS & METHODS

With in this study two RHE were used. In a first step a open-source reconstructed epidermis (OS-Rep) was used to calibrate the system and to derive basic performance standards[3]. Secondly the setup was adapted to an commercial available model produced by ATERA (ATERA-RHE model). To measure the electrical parameters of both RHE a measurement setup was established that allows a reproducible measurement of the complex electrical resistance at alternating current (impedance) in a frequency range between 1 Hz and 100 kHz (Fig. 1).

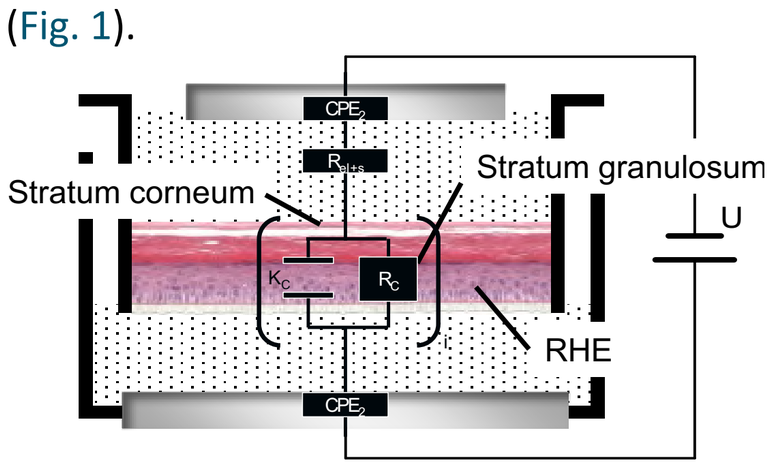

Figure 1: Experimental setup to measure the impedance of reconstructed human epidermis (RHE). The system can be described by the depicted equivalent circuit (CPE: constant phase element, Rel+s: Electrical resistance of the technical part, Kc: Capacitance of the RHE, Rc: Ohmic resistance of the RHE).

RESULTS

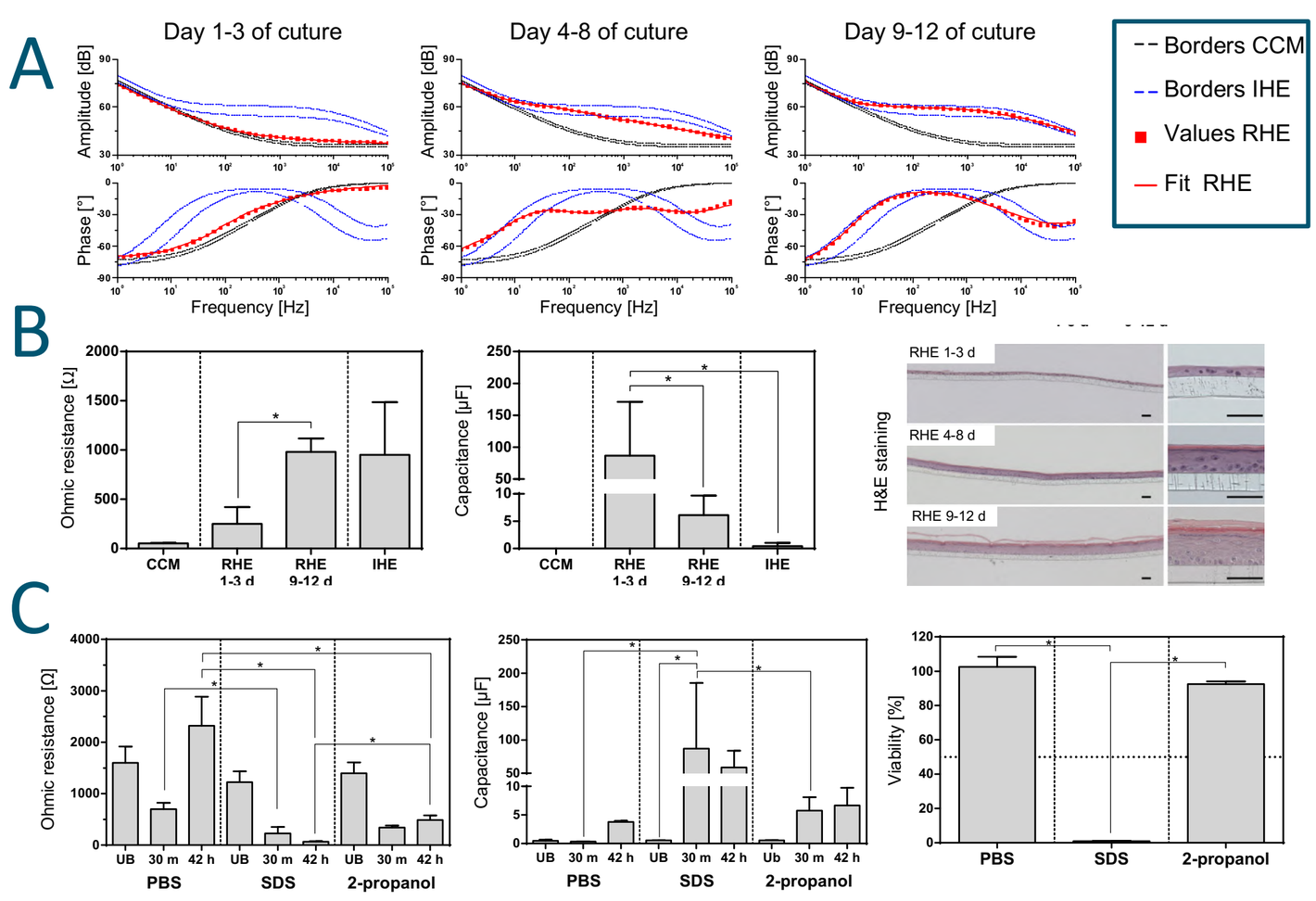

Figure 2 Impedance of OS-Rep model: A: Impedance spectra of the OS-Rep in comparison with un-seeded cell culture membranes (CCM) and isolated human epidermis (IHE). B: Extracted electrical parameters during epidermal differentiation in comparison with H&E stained RHE. C: ImpSpec as an additional endpoint in skin irritation testing. Values are compared to the result of a standard viability assay. (UB: Untreated RHE before the test, 30 m: RHE after the application of the test substances, 42 h: RHE after a 42 hour recovery phase)

OS-Rep exhibits characteristic impedance spectra in a frequency ranging between 1 Hz and 100 kHz, which is comparable to the spectra of freshly isolated human epidermal biopsies (IHE) (Fig. 2/A). From the spectra, we extracted electrical parameters of the OS-Rep such as the capacitance and the ohmic resistance. These parameters change significantly during epidermal differentiation (Fig. 2/B) and were used to quantify the effects of chemical disruption of the epidermal integrity (Fig. 2/C). Most relevant, ImpSpec shows a sufficient sensitivity to detect a transient decreased ohmic resistance caused by 2-propanol, which is classified as a non-irritant by standard skin irritation assays (Fig. 2/ D). Moreover, we used the system on the commercially available ATERA-RHE model and found comparable impedance spectra and impedance values (Fig. 3). Within these studies only little variations between different models could be observed demonstrating the high reproducibility of the model.

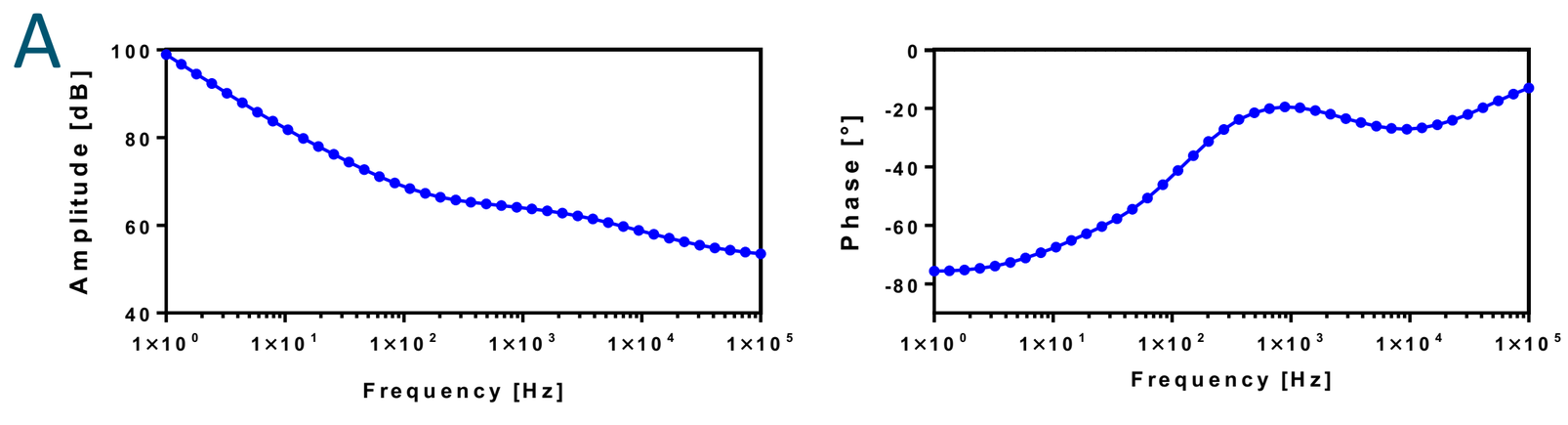

Figure 3 Impedance of the ATERA-RHE model: A: Impedance spectra of the ATERA-RHE model B: Ohmic resistance of the ATERA-RHE model at day 14 and day 17.

CONCLUSION

In our work we could create a new technology for the analysis of tissue-models, which is a vital requirement to increase the success of in-vitro-test-methods. Furthermore, our results indicate that ImpSpec might be applicable as an additional quality criteria for RHE and a method to assess mild irritative effects.

REFERENCES

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19th International Congress on In Vitro Toxicology ESTIV– ***Juan-les-Pins- October 17 – 20, 2016***

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