

Development and optimization of a 3D skin construct

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Master thesis, Therapeutic Technologies

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INTRODUCTION

The aim of the study was to develop and optimize a 3D construct derived from human keratinocytes. This would allow to mimic *in vivo* human skin as closely as possible in architecture, composition, and lipid organization and could serve as skin substitute for dermal and transdermal drug application.

CONCEPT

The stratum corneum (SC), the outermost layer of the skin, is responsible for its barrier function. It is often compared with a brick-and-mortar-like structure, where the corneocytes are the bricks and the lipid matrix the mortar [1], which consists of ceramides (CER), free fatty acids (FFAs), and cholesterol (CHOL) [2]. The route through this lipid matrix is considered to be the predominant pathway [3].

The constructs were established by using combinations of different media, drying and cultivation procedures. They were tested against pig ear skin in permeation studies with a hydrophilic (caffeine) and a lipophilic (flufenamic acid) compound.

The extent of stratification was observed by histology sections while the composition was determined by HPTLC (high performance thin layer chromatography). The characterization on an ultra structural level was done using ATR-FTIR (attenuated total reflectance Fourier transform infrared spectroscopy) and SAXS (small angle X-ray scattering). While ATR-FTIR provides information about the lateral packing, SAXS provide information on the lamellar packing.

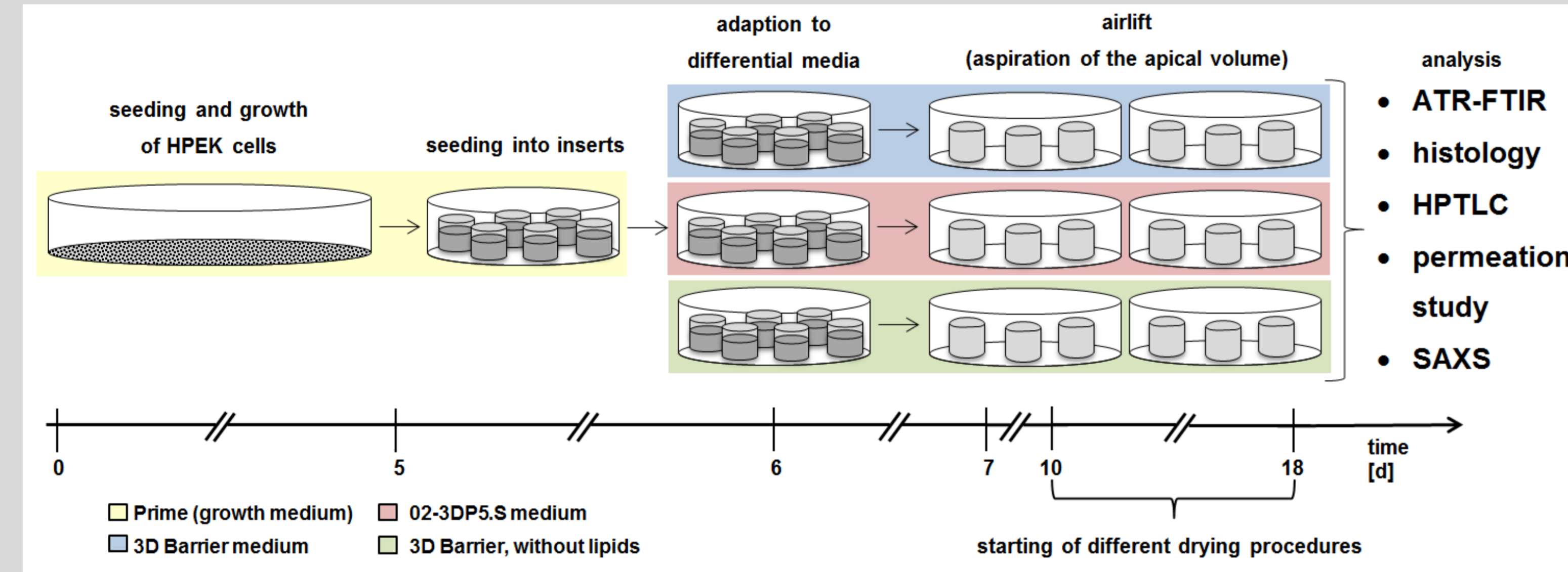


Fig. 1: Schematic representations of HPEK construct preparation. On day seven, the apical media was aspirated (airlift). On day 18 the constructs were fully established and ready for the corresponding analysis.

The work was carried out in collaboration with the company CELLnTEC Advanced Cell Systems AG located in Berne, Switzerland, who is the distributor of this 3D skin construct kits and the corresponding media.

RESULTS

The permeability values for all constructs exceeded that of the reference (pig ear skin) clearly, whereas the construct, which was cultivated in O2-3DP5.S medium, was found to be the least permeable. None of the drying procedures contributed to a better skin barrier. Only a prolonged cultivation of additional seven days led to an improvement concerning permeability.

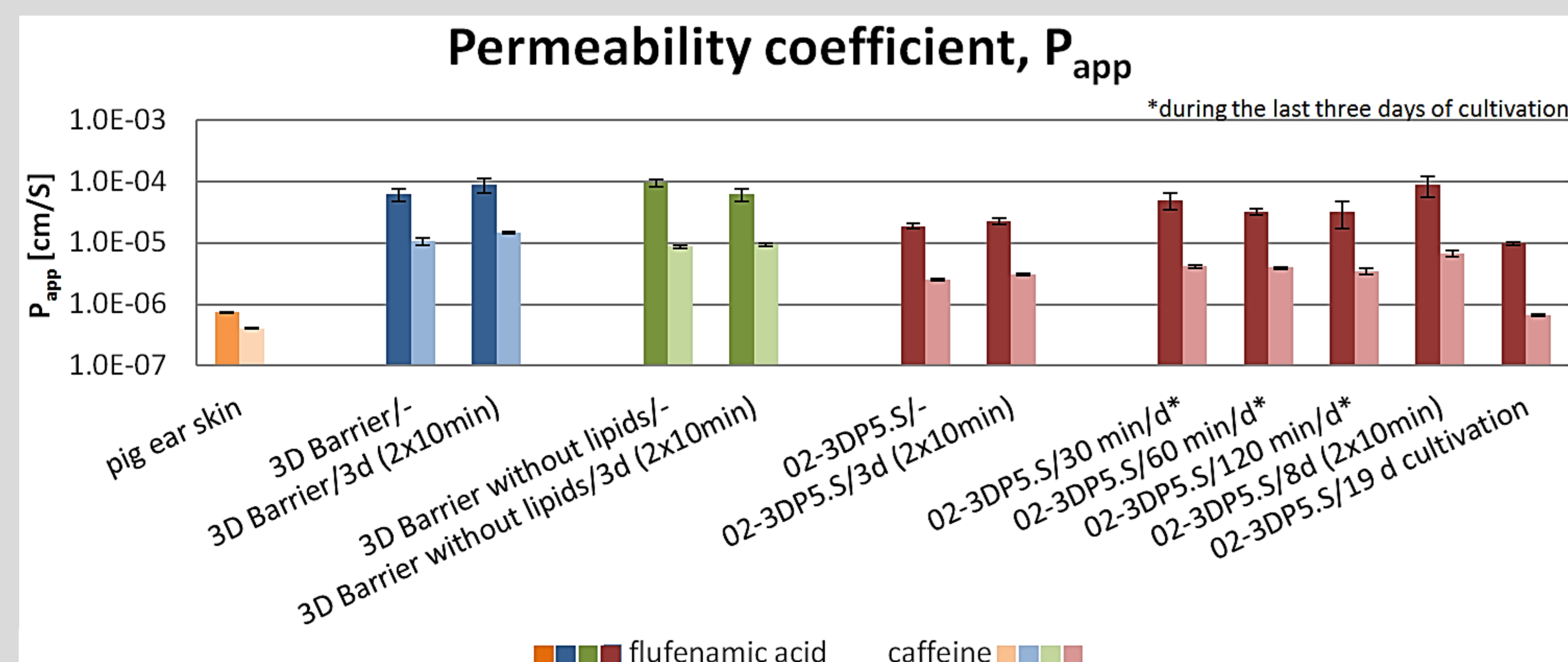


Fig. 2: P_{app} values \pm standard error for caffeine and flufenamic acid calculated with the software EasyFit.

The histology analysis showed that the overall structure with all epidermal layers was formed. No obvious differences, induced by media composition, were seen between the constructs. The stronger the drying was the thinner the construct became. The prolonged cultivation resulted in similar overall thickness but the SC layer was 3.5 times thicker compared to pig ear skin.

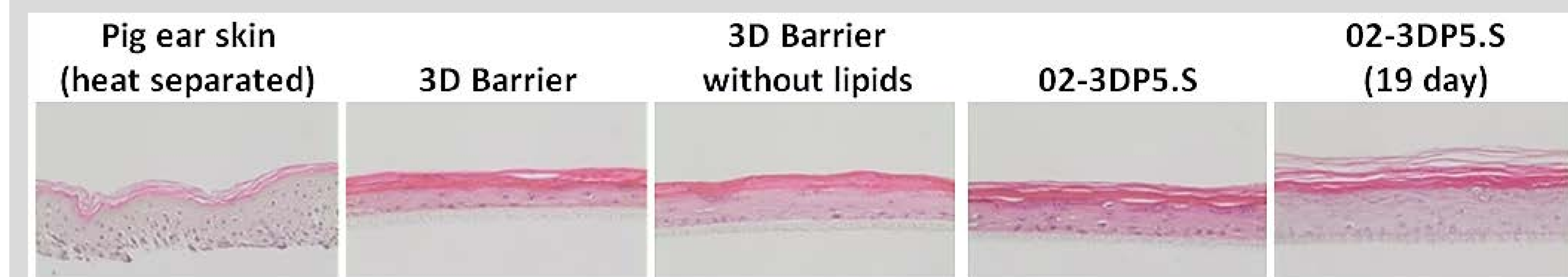


Fig. 3: Histological structure of 3D skin constructs from hematoxylin-eosin stained paraffin sections.

The ATR-FTIR measurements revealed that only pig ear skin contains the more ordered orthorhombic lipid packing, whereas the constructs composed of a hexagonal or a liquid phase are therefore more permeable.

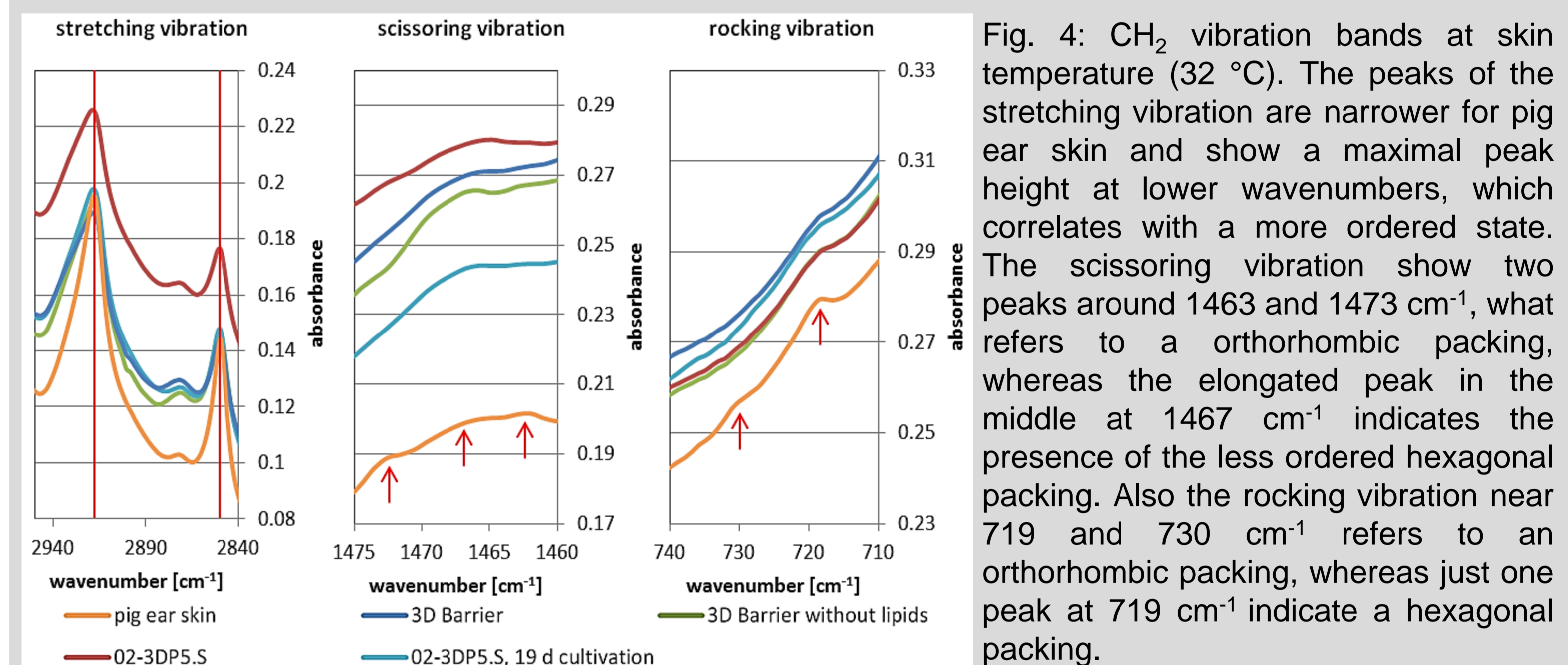


Fig. 4: CH_2 vibration bands at skin temperature (32 °C). The peaks of the stretching vibration are narrower for pig ear skin and show a maximal peak height at lower wavenumbers, which correlates with a more ordered state. The scissoring vibration show two peaks around 1463 and 1473 cm^{-1} , what refers to a orthorhombic packing, whereas the elongated peak in the middle at 1467 cm^{-1} indicates the presence of the less ordered hexagonal packing. Also the rocking vibration near 719 and 730 cm^{-1} refers to an orthorhombic packing, whereas just one peak at 719 cm^{-1} indicate a hexagonal packing.

The interpretation of SAXS measurements were challenging and no definitive statements can be made because peaks of skin samples are usually much broader, overlapped, and less intensive compared to pure lipid mixtures in which proteins are absent [4].

CONCLUSION

The barrier integrity was reduced in all constructs compared to pig ear skin. Only O2-3DP5.S medium provided comparable results concerning permeability behavior whereas none of the drying procedures led to further improvements. Although a prolonged cultivation (19 day) provided P_{app} values close to pig ear skin, the SC layer was 3.5 times thicker and did therefore not meet *in vivo* situation. Possible explanations for a reduced barrier function can either be a higher degree of conformational disordered lipids in the constructs [5] due to a reduced chain length of the FFAs [6] or an increased level of monounsaturated fatty acids. But also an incomplete ceramide synthesis, found in HPTLC analysis, contributed to this situation. To counter this, media supplementations with increasing amounts of FFAs could improve the barrier function. With other supplementations, for example vitamin C, the hydroxylation of long chain fatty acids is stimulated which is accompanied with a complete ceramide synthesis [7].

Hence, the established construct cannot yet fully replace human skin for dermal and transdermal drug application experiments. But with further improvements it becomes a good alternative mainly for hydrophilic compounds.

REFERENCES

- [1] Elias, P. M. (1983), *J Invest Dermatol*, 80(1 Suppl), 44s-9s.
- [2] Gray, G. M. and Yardley, H. J. (1975), *J Lipid Res*, 16(6), 434-40.
- [3] Albery, W. J. and Hadgraft, J. (1979), *Journal of Pharmacy and Pharmacology*, 31(3), 140-147.
- [4] Bouwstra, J. A. et al. (1995), *Journal of Lipid Research*, 36(4), 685-695.
- [5] Thakoersing, V. S. et al. (2012), *Tissue Eng Part C Methods*, 18(1), 1-11.
- [6] Janssens, M. et al. (2009), *Biochimica Et Biophysica Acta-Biomembranes* 1788(3), 732-742.
- [7] Ponc, M. et al. (1997), *J Invest Dermatol*, 109(3), 348-55.