Cosmetics for everyone requires testing for all ages : creation of 3D Bioprinted old and young skin models for real efficacy testing

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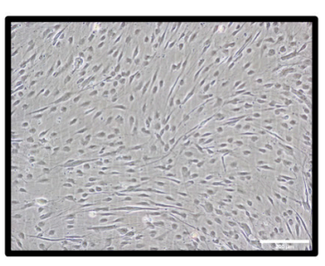
INTRODUCTION

The historical concentration of beauty marketing to the young and beautiful has thankfully given way to a broader fairer attention to women of all ages, and even men, with global cosmetics increasingly becoming a mainstream accepted globally. However, cosmetics for all ages is a challenge not to be underestimated. Young skin has significantly different characteristics to older skin. Thinning characteristics of older skin with changes in matrix protein glycosaminoglycan combinations, lower collagen and hyaluronic acid, damaged elastin and increased water loss from the barrier all lead to differences in how cosmetics react on older skin. The more sinusoidal nature of older skin in general and tendance towards wrinkles has important implications for how cosmetic ingredients are adsorbed to the skin. In addition, many of the OECD recommended tests are based on out of date manually constructed epidermal models, which more often than not contain pooled keratinocytes from many donors, with no regard for immunology at all.
For this reason, we have advanced our 3D bioprinted full thickness skin models towards creating a variety of age-related models, with same-donor cells (rare for in vitro skin models) to reproduce the level of thickness and sinusoidal behavior seen in real life with the aim to help advance efficacy testing.

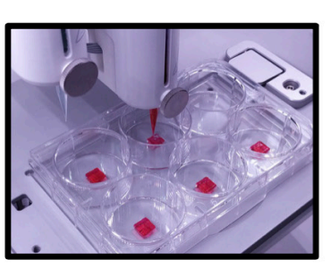
METHODS

AMPLIFICATION



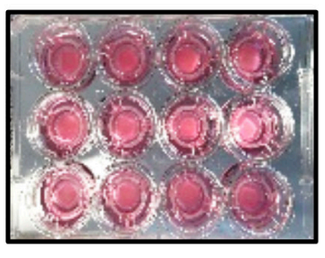
Human keratinocytes and fibroblasts were expanded from donations of human male and female skin donated following elective surgery and full ethical consent from donors ranging from 1 years old to 67 years old, both male and female.

3D BIOPRINTING



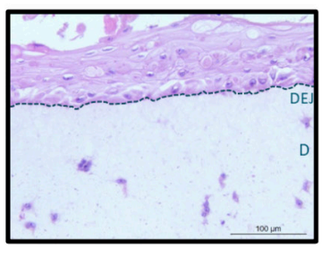
Cells were mixed with a bioink (CELLINK, Sweden) and cartridged into a CELLINK pneumatic 3D bioprinting system. Full thickness epidermal-dermal skin same-donor models were printed at different thicknesses and with additional or subtracted laminin and collagen substitution.

UV-INDUCTION



Printed models from younger donor were inducted with a UV-A light source device (CTIBiotech, Lyon, France 10J/cm², every day during 5 days). After irradiation, fresh culture medium was added and 3D models were incubated again at 37°C, 5% CO2 until the next treatment day.

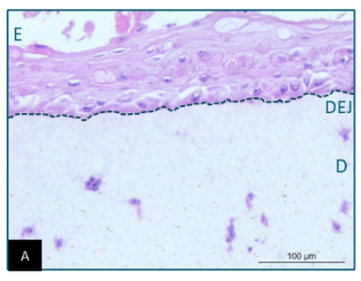
HISTOLOGY



All printed models were harvested for histological analysis with Hematoxylin, Eosin, Saffron, CD44, HAS1 and HAS2 stainings to evaluate structure and function of the models.

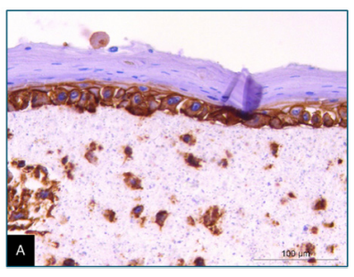
RESULTS

Young donor

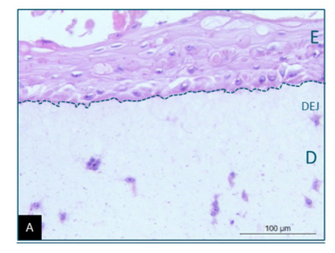


- **Good fibroblasts growth Thick epidermis,**
- **Differentiated from basal layer to stratum corneum**

Young donor

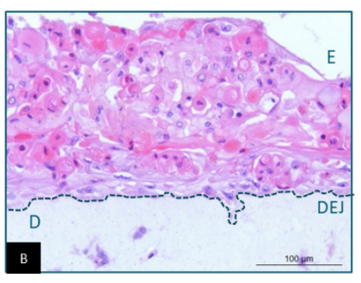


- **CD44 not expressed in cornified layer**
- **But expressed in basal layer and the dermal part (A)**



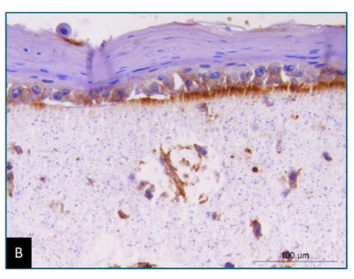
- **Sacrificial ink (pluronic) printed in the model increase the sinusoidal shape (B)**

Old donor

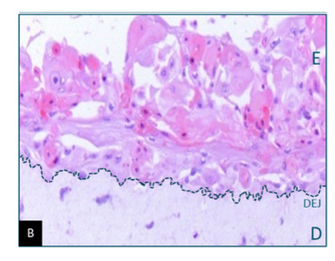


- **Good fibroblasts growth Thinner epidermis,**
- **Stratum corneum in formation**

Old donor

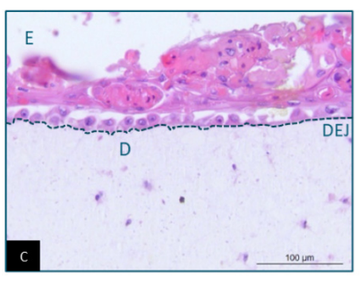


- **HAS1 expressed in the epidermis and dermis**
- **Not detected in cornified layer**
- **More expressed in dermo-epidermal junction (B)**



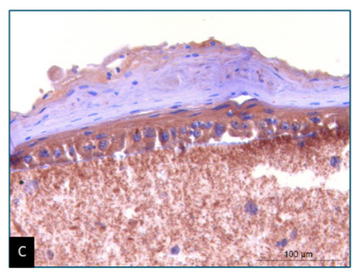
- **Sinusoidal shape in the dermal part : printing nozzle dived into lower layers (C) No significant**

Young donor induced

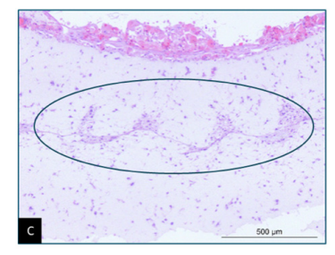


- **Damaged and thinner epidermis after UV treatment**
- **Anachronistic keratinocytes differentiation**

Young donor induced



- **HAS2 expressed in the epidermis and dermis**
- **Not detected in cornified layer**



- **Differences in the structure of the DEJ compared to the previous models**

CONCLUSION

- **Improvement and diversification of 3D reconstructed skin models is a key to better assess safety and efficacy ingredient assessment.**
- **To personalize and propose a broader range of age-related skin models, we demonstrated the ability of our reconstructed skin models to develop according to age of donor.**
- **Furthermore, testing possibilities are even diversified with functional UV stress photoaging models.**
- **We demonstrated the functionality of models to synthesize Hyaluronan, a key marker involved in skin ageing.**