Preservation of human skin integrity and metabolism during 10 days in culture with NativeSkin® system

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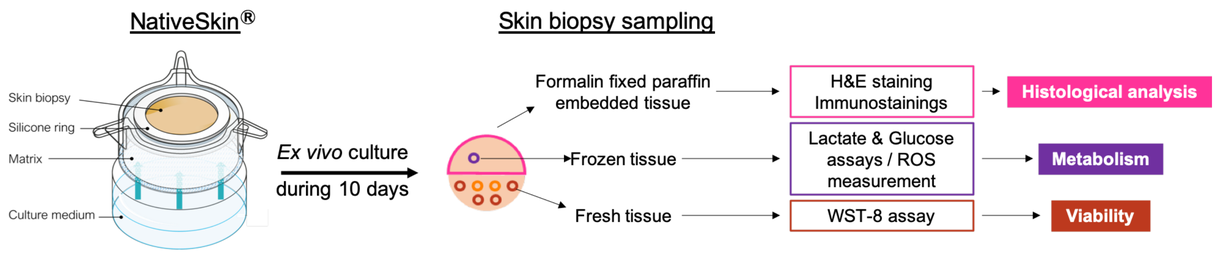
Human epidermis is not vascularized and oxygen only reaches epidermal cells by diffusing through the stratum corneum or from the dermal papillae which contains a dense capillary network. It is known that the main bioenergetic pathway of human epidermis is anaerobic glycolysis (Ronquist et al., 2003). Therefore, glucose consumption and a high production of lactate indicate an active metabolism in skin. This pathway has not been well investigated in human skin biopsies cultured *ex vivo*.

Genoskin has developed NativeSkin®, a standardized *ex vivo* human skin model containing both epidermis and dermis, produced from surgical skin samples. The skin model is embedded in a proprietary nourishing gel-like matrix with epidermal surface left in direct contact with the air. NativeSkin® represents a highly predictive and cost-effective last-line screening tool in laboratory conditions prior to *in vivo* clinical evaluations. We study here the integrity, viability, and metabolism of NativeSkin® models after 10 days of *ex vivo* culture.

Experimental Workflow

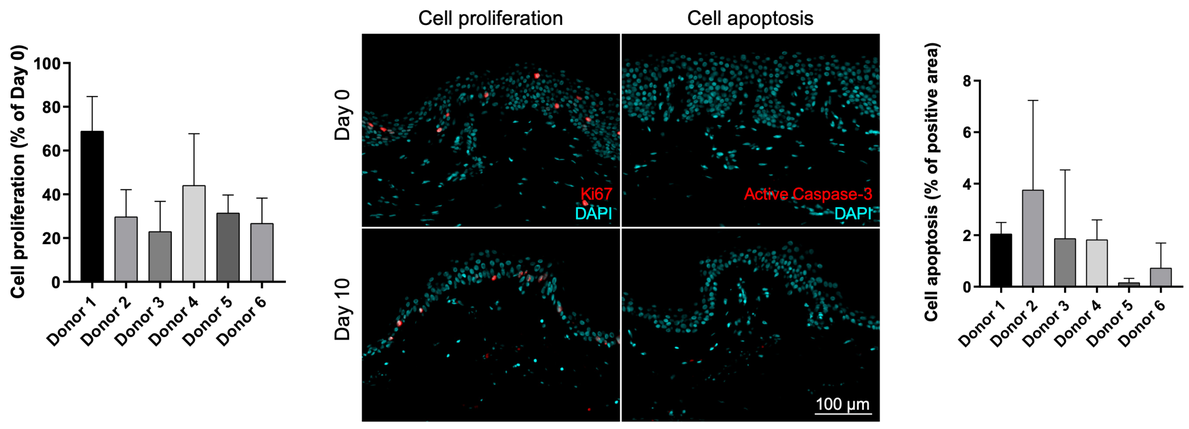


NATIVESKIN® MODEL CULTURE DURING 10 DAYS AND SAMPLING.

The experiment was performed on 7 donors. NativeSkin® M models (15 mm of diameter) were cultured for 10 days. Culture media was changed daily. At Day 0 and Day 10, skin models were sampled as described. Culture media was frozen at -80°C.

Donor #	Gender	Age	Anatomical site	Number of replicates
1	Female	42	Abdomen	n=2
2		28		n=2
3		58		n=4
4		37		n=4
5		37		n=4
6		38		n=2
7		52		n=2

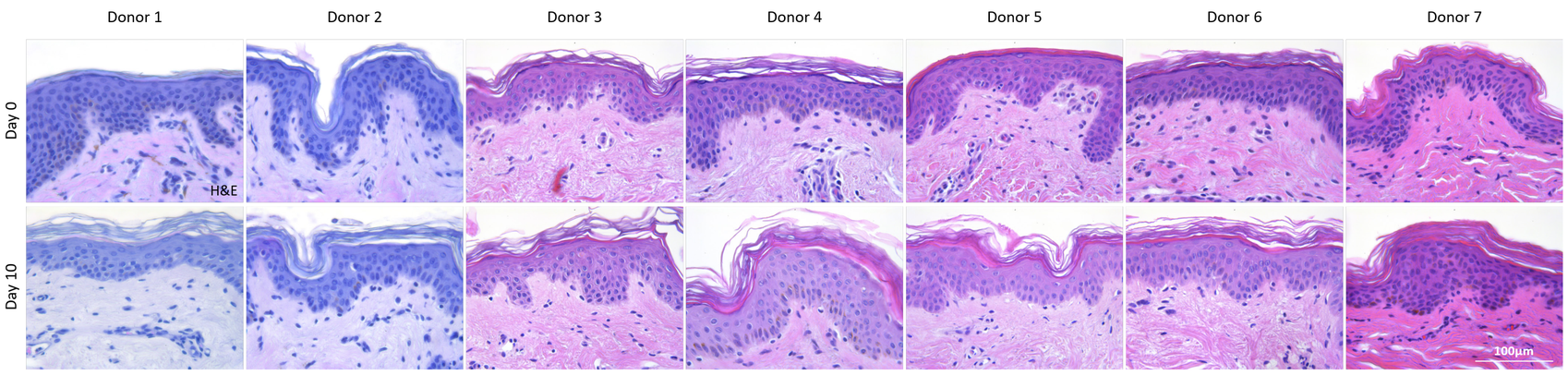
NativeSkin® shows cell proliferation and no apoptosis at day 10



ANTI-KI67 AND ANTI-ACTIVE CASPASE-3 IMMUNOSTAININGS AND QUANTIFICATION.

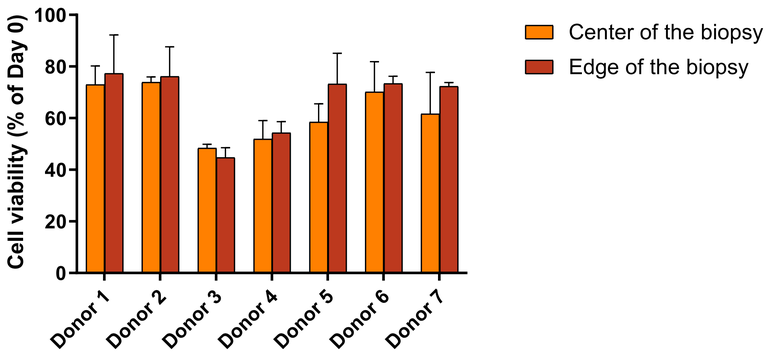
Immunostainings were performed on 5µm thick skin slices. Representative images at Day 0 and Day 10 are presented. Quantifications were performed on 6 representative pictures of the epidermis.

NativeSkin® maintains normal skin integrity and a significant cell viability at day 10



NORMAL SKIN INTEGRITY AFTER 10 DAYS OF *EX VIVO* CULTURE.

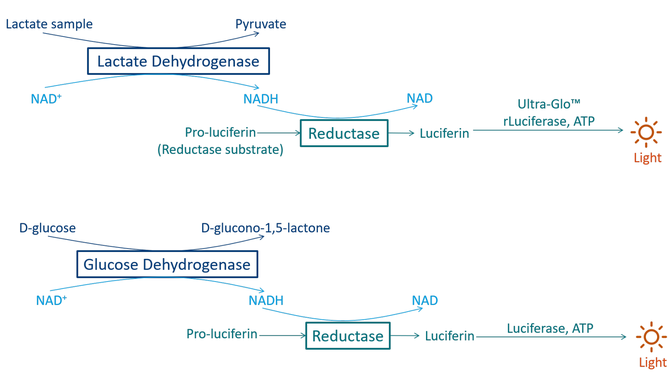
Hematoxylin and Eosin staining was performed on 5µm thick skin slices. A representative image of NativeSkin® model at Day 0 and Day 10 is presented for each donor.



CELL VIABILITY AFTER 10 DAYS *EX VIVO* CULTURE

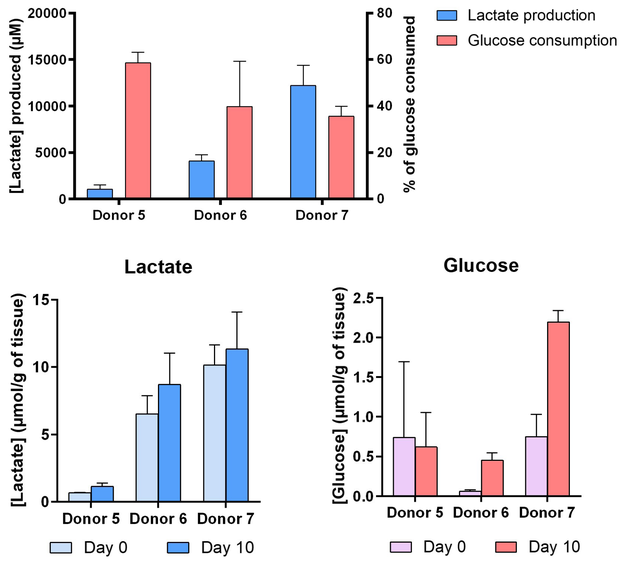
WST-8 assay was performed on small punch biopsies (2 in the center and 4 in the edge), in order to assess cell viability in different area of the model.

NativeSkin® consumes glucose and produces lactate at day 10



DOSING OF GLUCOSE AND LACTATE

We used Promega bioluminescent NADH assays, Glucose-Glo™ and Lactate-Glo™ Assays.
(Illustration based on Promega figure)



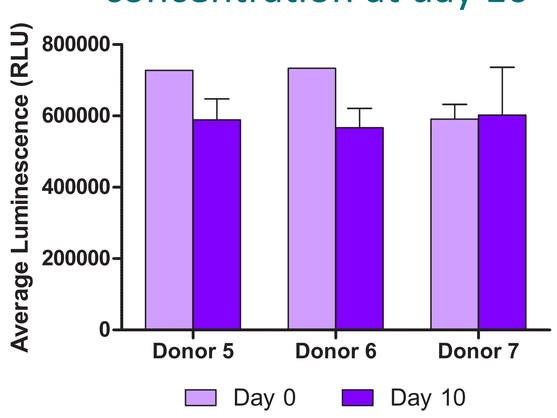
LACTATE PRODUCTION AND GLUCOSE CONSUMPTION IN CULTURE MEDIA.

Concentrations of lactate produced and glucose consumed in culture media between Day 9 and Day 10 were dosed. The bars represent the mean ± SD of replicates.

LACTATE PRODUCTION AND GLUCOSE CONSUMPTION

Lactate and glucose concentrations in NativeSkin® cultured 10 days were measured on 1 frozen biopsy for each sample.
The bars represent the mean ± SD of replicates.

NativeSkin® maintains normal ROS concentration at day 10



ROS CONCENTRATION IN NATIVESKIN® MODELS

H_2O_2 concentration in NativeSkin® cultured 10 days was measured on 1 frozen biopsy for each sample using ROS-Glo™ H_2O_2 Assay (Promega). The bars represent the mean +/- SD of replicates.

NativeSkin® is an *ex vivo* human skin model that can normally be maintained in standard culture conditions up to 7 days when shipped to customers. Here, we demonstrate for the first time maintaining the skin model 10 days in *ex vivo* culture while preserving normal skin integrity, significant cell viability, proliferative cells and low levels of apoptosis.

We demonstrate that the skin metabolism of NativeSkin® at 10 days is comparable to fresh skin by measuring glucose consumption and lactate production using Promega's Glucose-Glo™ and Lactate-Glo™ Assays. We also demonstrate that NativeSkin® maintains normal ROS concentration after 10 days of *ex vivo* culture, using Promega's ROS-Glo™ H_2O_2 Assay. Our next step will be to evaluate the activity of specific human skin cytochromes involved in metabolization of drugs topically applied on NativeSkin®.