Improved animal component free skin medium for long term maintenance of human skin explants and assessment of dermal toxicity risks

A. Jamin[1], R. Li[1], S. Martin[2], S. Rohou[2], C. Pasdelou[2], H. Dacq[1], L. Oger[1], C. Cuddapah[2], RR. Friis[2], A. Sharma[1], C. Chesne[1]

[1]Biopredic International, PA de la Bretèche, Bldg A4, 35760 Saint-Grégoire, France
[2]Eurosafe, PA de la Bretèche, Bldg B1, 35760 Saint-Grégoire, France
[3]Curio Biotech, Rottenstrasse 7, 3930 Visp, Switzerland

Background: Different skin models, such as 3D-reconstructed skin or standard 2D monolayer cell culture, have been used for biological skin studies and for ingredient or finished product testing. The major limitation of those models is the lack of the skin native complex architecture because of containing at best 3 cell types. Native skin explants remain a relevant physiological model that best mimic the in-vivo situation, but they can only be cultivated for a short-term period at 32°C in a standard culture medium. Therefore many efforts were made on developing an improved culture medium for long term maintenance of skin explant cultures and for prediction of skin irritation, corrosion, sensitization and metabolism.

Objective: We aimed at developing a novel animal component free skin culture medium 1) for long-term culture skin explant with an up to a 5 day time of transport/storage at 4°C followed by a 14 day time at 32°C, with good skin histology integrity and physiological functions; 2) for an optimized *in vitro* skin culture model to predict dermal irritation.

Study Design

Day 0: skin tissue with superficial stretch marks from abdominoplasties of 4 female donors

Shipping at 4°C

Day 1: skin explant preparation
full thickness skin discs (∅ 10mm)

w/o transport/storage period

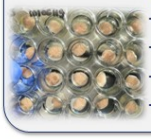
With hypothermic storage/transport period (2-5 days)

Day 2 to 14: Skin explants cultures in two skin media at 37°C

Optimized LT medium	D2	D7	D9	D14
Classic medium	D2	D7	D9	D14

5 time points: 24-well plates: 1 explant/400 µL of medium/well, medium changed every 2-3 days

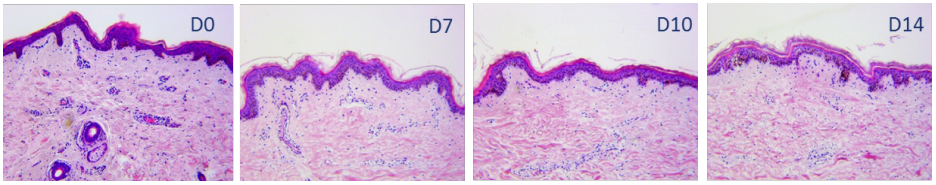
Performance evaluation of 2 skin culture media



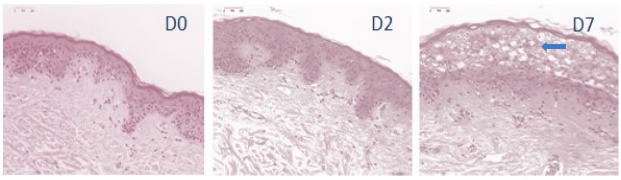
- Skin structure by H&E staining
- Skin viability and SDS irritation test by MTT assay
- Epidermis/dermis dissociation test with pliers
- TEER measurement

Prolonged structure integrity and viability of skin explant culture in optimized medium up to 14 days

Skin explant cultures in optimized medium

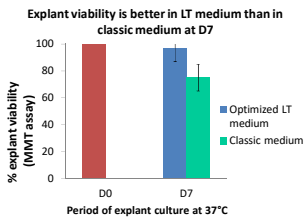


Skin explant cultures in classic medium

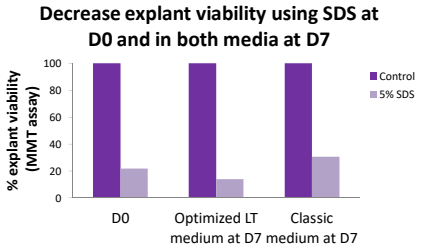


Histological evaluation of skin explant integrity by H&S staining

Degraded stratum spinosum of epidermis



Long term skin culture medium is suitable for irritation evaluation



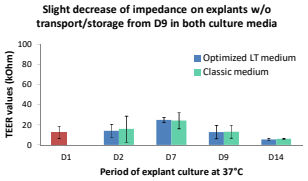
Well-preserved epidermis-dermis junction in optimized long term medium

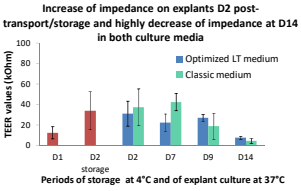
Dissociation test	w/o shipment (D1)		After 2 days of shipment		After 5 days of shipment	
Impact of storage/transport	☑☑☑		☑☑☑		☑☑☑	
Day of skin explant culture	Optimized LT medium	Classic medium	Optimized LT medium	Classic medium	Optimized LT medium	Classic medium
D2	☑☑☑	☑☑☑	☑☑☑	☑☑☑	☑☑☑	☑☑☑
D7	☑☑☑	☑☑☒	☑☑☑	☑☒☒	☑☑☑	☒☒☒
D9	☑☑☑	☒☒☒	☑☑☑	☒☒☒	☑☑☒	☒☒☒
D14	☑☑☑	☒☒☒	☑☑☑	☒☒☒	☑☑☒	☒☒☒

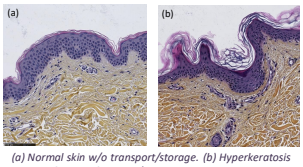
Dissociation test (n=3 explant values/end point):
☑ Epidermis-dermis well attached; ☒ Detachment of the epidermis from the dermis

- The optimized LT medium showed the best preservation of skin layer junctions in all tested conditions.
- The D5 of storage/transport impacted the epidermis-dermis junction by reducing the integrity of explant culture from D9 using the optimized medium.

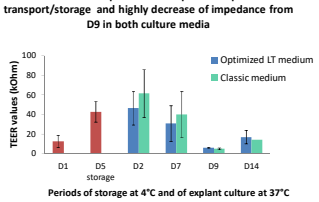
Impedance variations during transport/storage and culture in both media







(a) Normal skin w/o transport/storage. (b) Hyperkeratosis after D5 of storage/transport. H&E staining



Conclusions: We developed a new skin long term medium without animal component and observed good performances with prolonged viability of skin explants and well-preserved epidermis-dermis junction in the improved medium up to 14 days, in comparison to that of the classical medium. A good predictivity of induced irritation was also demonstrated up to 7 days by MTT assay. We will further investigate irritation, as well as sensitization and metabolism in skin explant culture until day 14.
This improved long term skin explant model is promising for long term cosmetic product assays including the chronic toxicity testing.