# Evaluation of Lesions Caused by Hydrofluoric Acid (HF) on Human Skin *ex vivo* and Decontamination With Tap Water + Calcium Gluconate or Hexafluorine®

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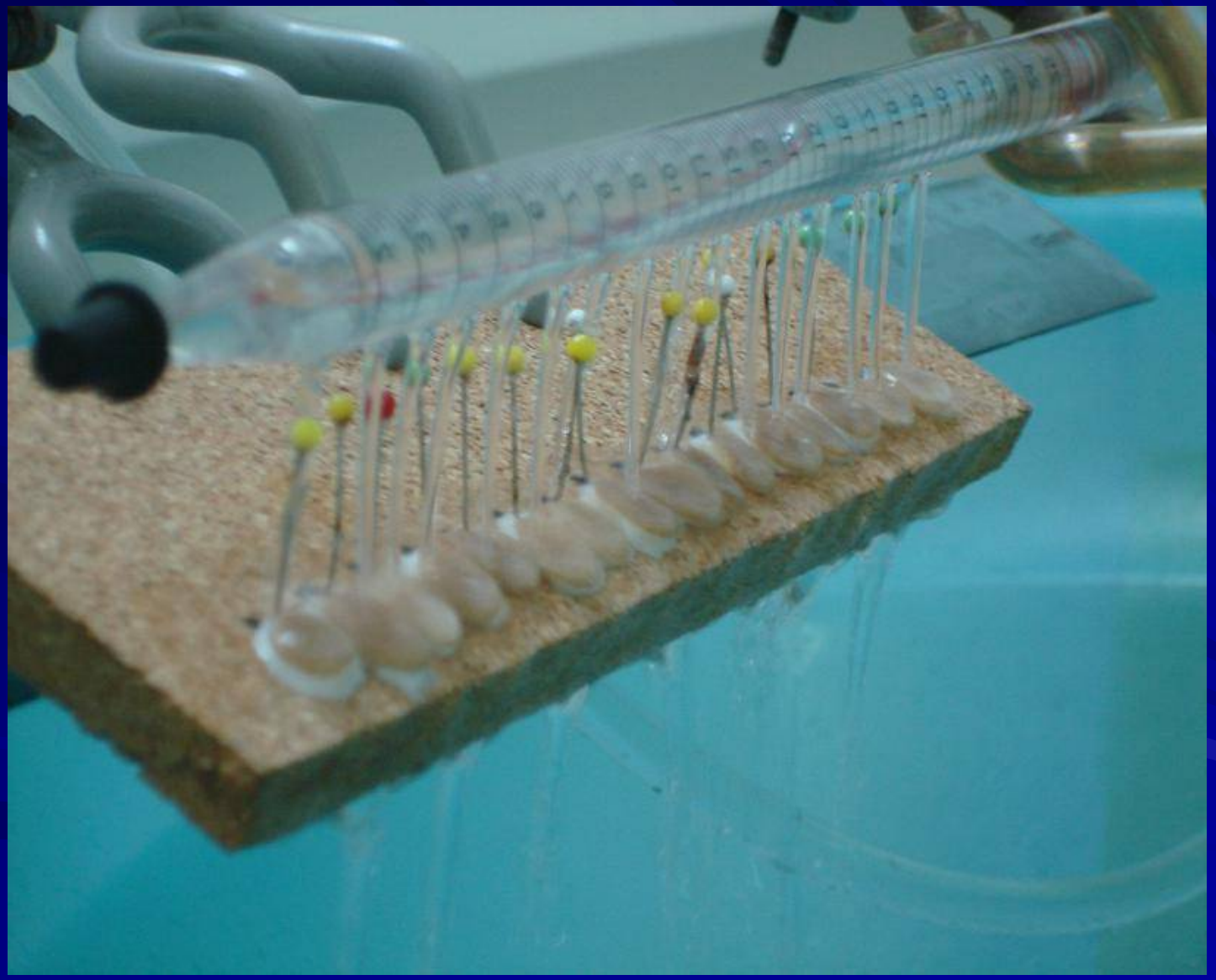
# Summary

- Human skin explants *ex vivo* represent:
  - A means of testing hazardous substances and decontamination measures
  - *Without* using live animal models
  - In cases where studies in living humans are not
    - *Ethically feasible*
    - *Technically feasible*

# Human Skin Explants *ex vivo*

- The BIO-EC Laboratory, Clamart, France has developed:
  - A technique for ethically using human skin explants
  - Maintained alive *ex vivo*
  - For the study of caustic or irritant substances and their decontamination

# Human Skin Explants



# Human Skin Explants *ex vivo* *Methods*

- Products
  - HF:  furnished by Study Sponsor; FLUKA, reference 47610; Lot No. 7125A; exact concentration = 73%
  - Tap Water
  - Calcium gluconate 2.5% gel; KAYS Medical, Lot No. 2001/09
  - Hexafluorine®; Prevor Laboratory; Mini-DAP 200 mL; Lot No. F971201C; Expiration Date 04/03/2009

# Human Skin Explants *ex vivo* *Methods – Control Explants*

- 21 control Human skin explants, diameter approximately 10 mm, prepared from an abdominoplasty of a 45-year-old woman
- *After* obtaining informed consent
- Explants preserved alive in BEM (BIO-EC's Explant Medium)
  - At 37° C
  - Humidified atmosphere containing 5% $CO_2$

# Human Skin Explants *ex vivo*
## *Methods – Control explants*

- The 21 control explants were divided into 7 groups, 3 explants per group, with 70% HF exposure for:
  - 20 seconds
  - 1 minute
  - 2 minutes
  - 3 minutes
  - 4 minutes
  - 5 minutes

# Human Skin Explants *ex vivo* *Methods*

- 70% HF applied
  - Soaked into filter paper disk
  - Chosen to take up 30 µL HF

# HF Application

# Human Skin Explants *ex vivo*
# *Methods – Control Explants*

- As compared with explants with ***no*** HF application:
  - ***20 Seconds contact***: *no alterations* of epidermis or dermis
  - ***1 minute contact***: *slight alterations* in the upper epidermal layer
  - ***2 minutes contact***: *Clearly increased alterations* in the upper epidermal layer
  - ***3 minutes contact***: *obvious lesions* in the epidermal layer
  - ***4 minutes contact***: *very clear lesions* in the epidermal layer
  - ***5 minutes contact***: *obvious lesions* in the epidermis and papillary dermis

  - **In these experimental conditions, the initial cellular alterations are observed after 2 minutes of contact with HF**

# Human Skin Explants *ex vivo* *Methods - Decontamination*

- 86 Human Skin explants, approximately 10 mm diameter, obtained from an abdominoplasty of a 35-year-old woman
- *After* obtaining informed consent
- Preserved alive in BEM medium
  - At 37° C
  - Humidified atmosphere with 5% $CO_2$

# Human Skin Explants *ex vivo* *Methods - Decontamination*

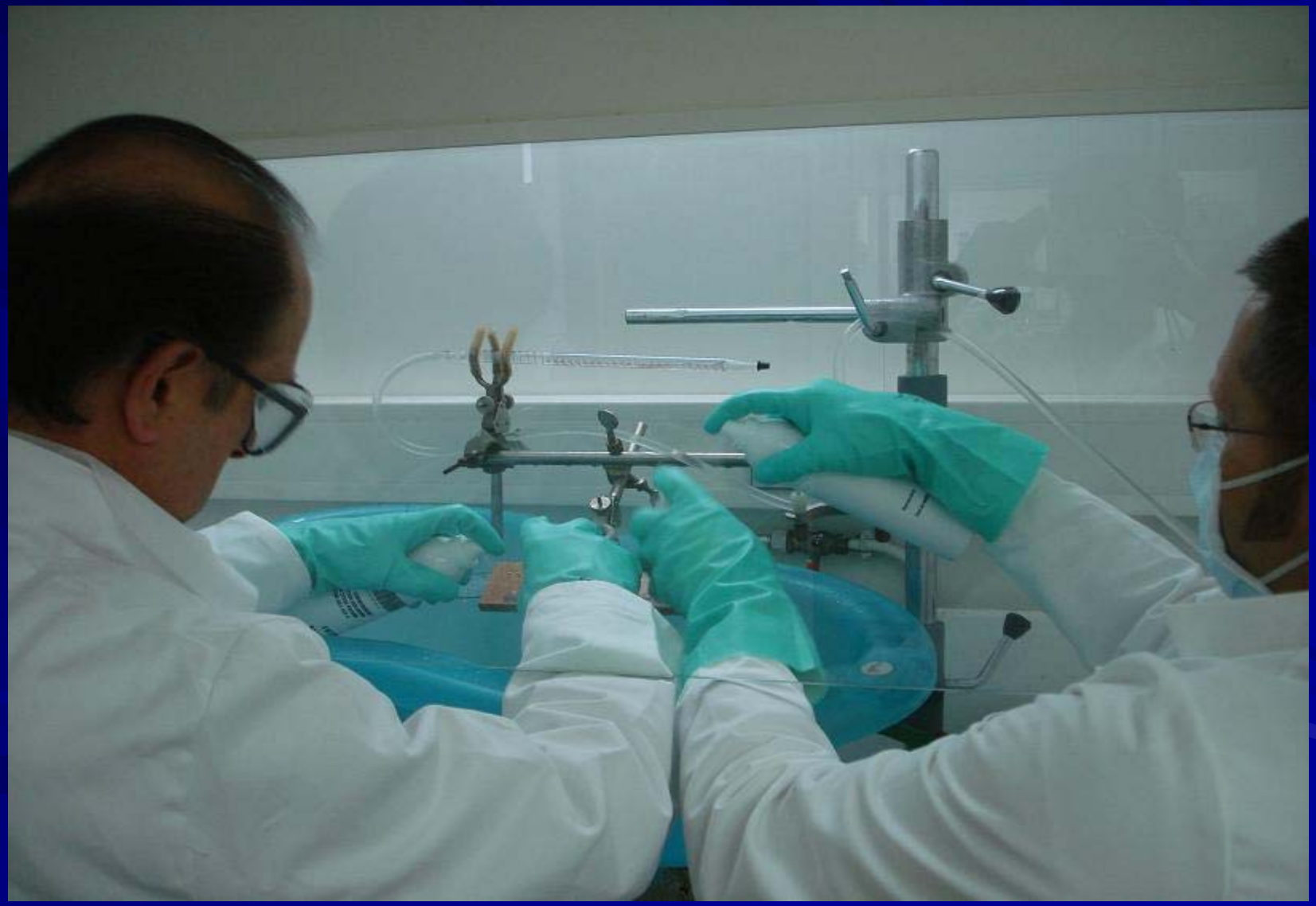
- Explants divided into 5 groups:
  - ***Group 1***: 20 explants; (*controls*, ***no*** HF application)
  - ***Group 2***: 18 explants: 70% HF, 20 seconds contact (*untreated controls*)
  - ***Group 3***: 16 explants 70% HF, 20 seconds contact (*Tap Water washing 15 minutes + Calcium Gluconate Gel 1 g/cm$^2$*)
  - ***Group 4***: 16 explants 70% HF, 20 seconds contact (*washing with 200 mL Hexafluorine® over approximately 5 minutes*)
  - ***Group 5***: 16 explants 70% HF, 20 seconds contact (*washing with 2 x 200 mL Hexafluorine® -- 400 mL total -- over approximately 10 minutes*)

# Human Skin Explants *ex vivo* *Methods - Decontamination*

- Histopathology Sampling Times:
  - ***Group 1:*** T0, 20 seconds, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours
  - ***Group 2:*** 20 seconds, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours
  - ***Group 3:*** After end of washing: 5 minutes,10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours
  - ***Group 4:*** After end of washing: 5 minutes,10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours
  - ***Group 5:*** After end of washing: 5 minutes,10 minutes, 15 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours

# Tap Water Washing

# Hexafluorine® Application



# Human Skin Explants *ex vivo*
# *Results - Histopathology*

- ***Untreated explants:***
  - Independent of the length of explant survival, no modifications of the epidermal or dermal structures observed.

# Human Skin Explants *ex vivo*
## *Results - Histopathology*

- ***70% HF-exposed explants, no decontamination:***
  - ***Clear alterations in the epidermis and papillary and inferior reticular dermis at 5 & 10 minutes***
  - ***Clearly increased alterations after 15 & 30 minutes and 1 hour***
  - ***Epidermal structures clearly necrotic at 24 hours***

# Human Skin Explants *ex vivo*
# *Results - Histopathology*

- ***70% HF-exposed explants washed with Tap Water + Topical Calcium Gluconate Gel:***
  - After 5 & 10 minutes, ***no alterations*** of the epidermis or dermis
  - After 15 minutes, ***very clear cellular alterations*** in the epidermis and papillary and inferior reticular dermis
  - The alterations ***decrease*** after 30 minutes, but are ***more definitively observed*** after 1 & 2 hours
  - ***Slight edematous lesions*** observed in the epidermal basal layer after 4 hours which ***clearly increase*** after 24 hours

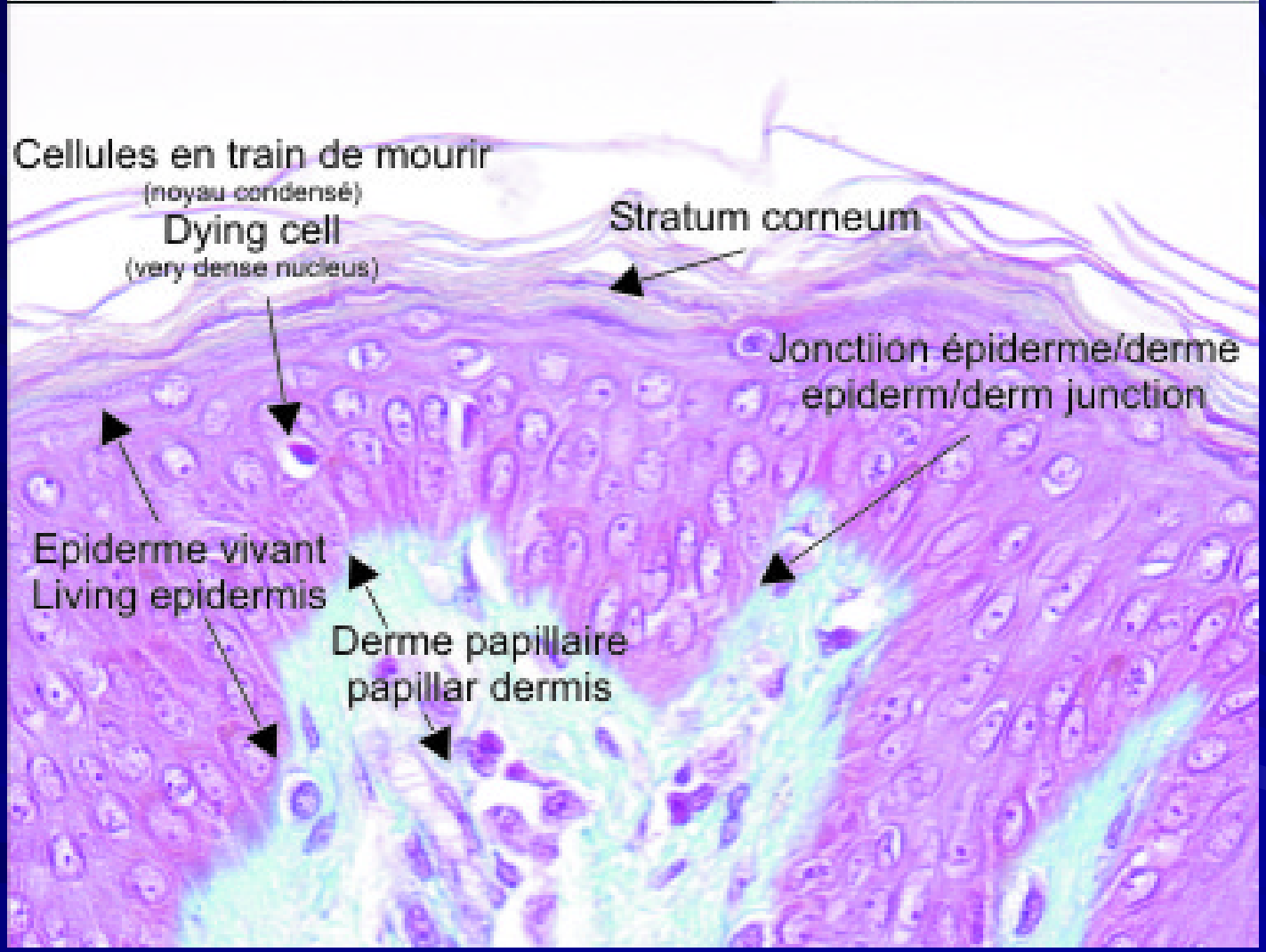
# Human Skin Explants *ex vivo*
# *Results - Histopathology*

- ***70% HF-exposed explants washed with Hexafluorine® 200 mL:***
  - After 5 minutes, some clear epidermal and dermal alterations in a single explant; ***none*** in other explants
  - After 10, 15, & 30 minutes, ***no alterations*** in the epidermis or dermis
  - After 1 & 4 hours, very clear epidermal and dermal alterations in a single explant; ***none*** in other explants
  - After 2 & 24 hours, ***no alterations*** in the epidermis or dermis

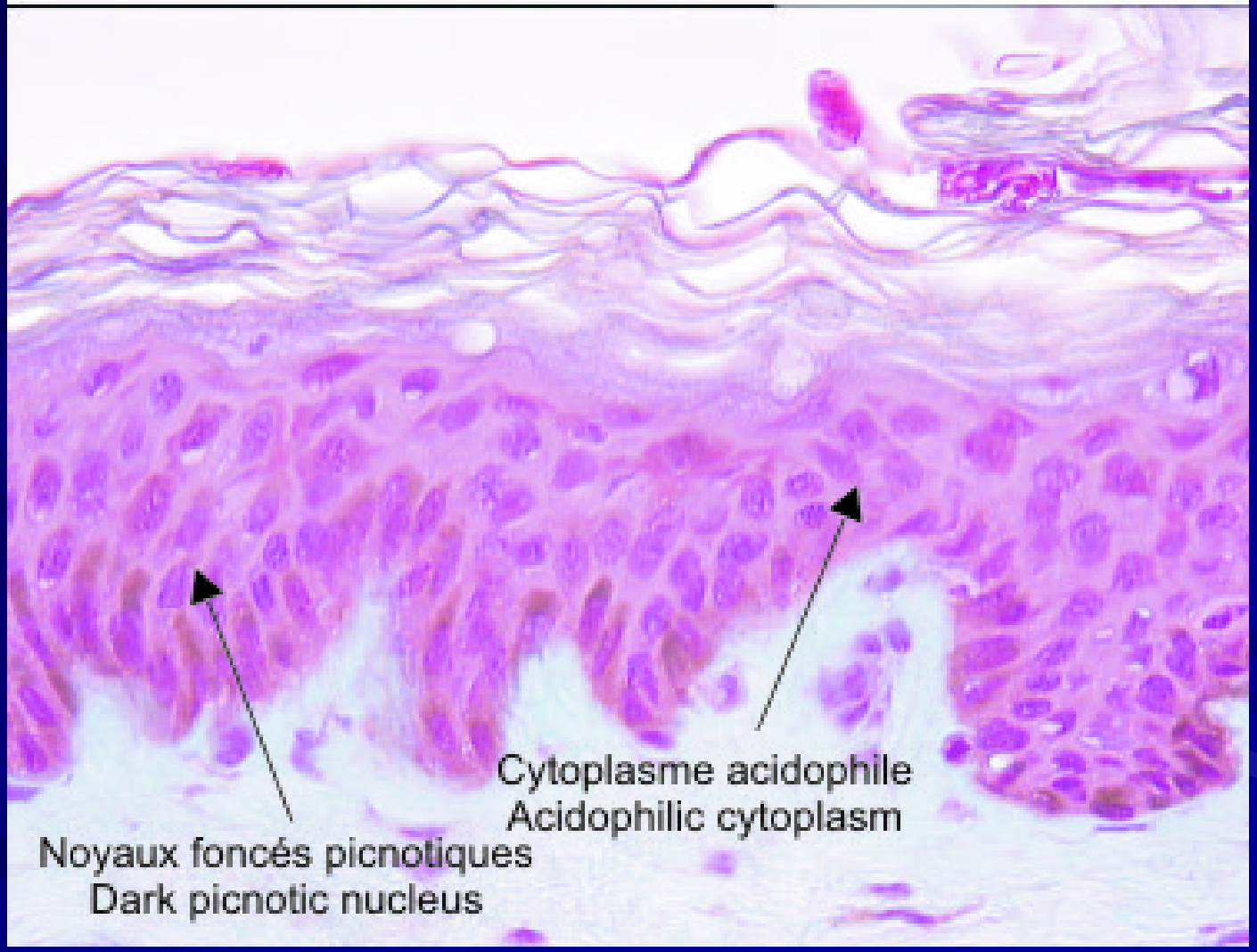
# Human Skin Explants *ex vivo* *Results - Histopathology*

- ***70% HF-exposed explants washed with Hexafluorine® 200 mL x 2 (400 mL total):***
  - After 5, 10 & 30 minutes and 1, 2, 4, & 24 hours:
    - ***No alterations of the epidermal and epidermal structures were observed***

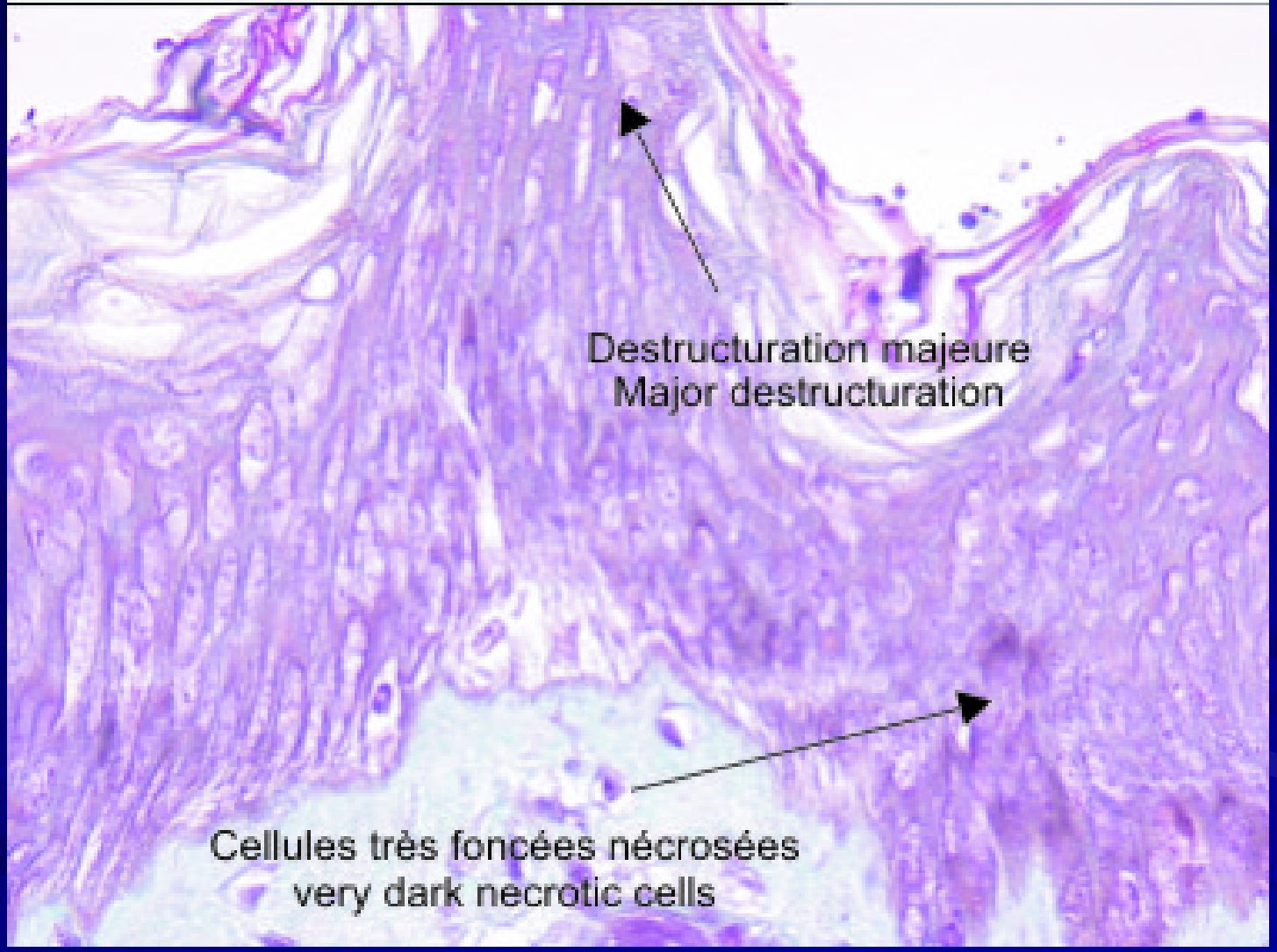
## EXPLANT NON EXPOSE MORPHOLOGIE GENERALE
## Non exposed explant general aspect



## EPIDERME HF 5 MIN DE CONTACT
## EPIDERMIS 5 MIN HF CONTACT



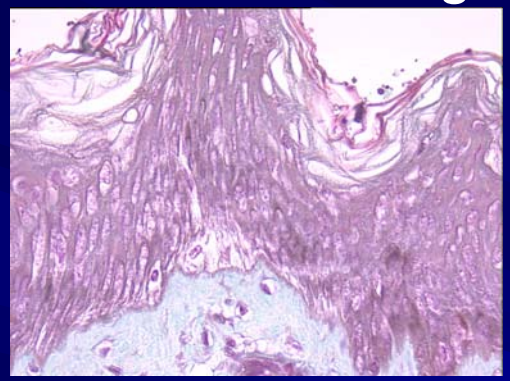
# HF 24 H DE CONTACT NECROSE COMPLETE
# HF 24 H CONTACT TOTAL NECROSIS



| | | Control group<br>No exposure non washing<br>(20 explants) | HF<br>without washing<br>(18 explants) | HF<br>Water washing + Calcium Gluconate<br>(16 explants) | HF<br>Hexafluorine® washing 400 ml<br>(16 explants) |
|---|---|---|---|---|---|
| **T0** | Epidermis | GM<br>= Good Morphology | | | |
| | Papillary dermis | GM | | | |
| | Reticular dermis | BM | | | |
| | | | | | |
| **20 s** | Epidermis | GM | | | |
| | Papillary dermis | GM | | | |
| | Reticular dermis | GM | | | |
| Total time after exposure | | 20 s | | | |
| **5 min** | Epidermis | GM | PN + AC | GM | GM |
| | Papillary dermis | GM | PN + AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 5 min | 5 min | 15 + 5 = 20 min | 10 + 5 = 15 min |
| **10 min** | Epidermis | GM | PN | GM | GM |
| | Papillary dermis | GM | AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 10 min | 10 min | 15 + 10 = 25 min | 10 + 10 = 20 min |
| **15 min** | Epidermis | GM | PN + AC | PN + AC moderate | GM |
| | Papillary dermis | GM | PN + AC | PN + AC | GM |
| | Reticular dermis | GM | PN + AC | PN + AC | GM |
| Total time after exposure | | 15 min | 15 min | 15 + 15 = 30 min | 10 + 15 = 25 min |
| **30 min** | Epidermis | GM | PN + AC | Some necrosed cells | GM |
| | Papillary dermis | GM | PN + AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 30 min | 30 min | 15 + 30 = 45 min | 10 + 30 = 40 min |
| **1 h** | Epidermis | GM | PN + AC | GM | GM |
| | Papillary dermis | GM | PN + AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 1 h | 1 h | 1 h + 15 = 1 h 15 | 1 h + 10 = 1 h 10 |
| **2 h** | Epidermis | GM | PN + AC | GM | GM |
| | Papillary dermis | GM | PN + AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 2 h | 2 h | 2 h + 15 = 2 h 15 | 2 h + 10 = 2 h 10 |
| **4 h** | Epidermis | GM | PN + AC | Slightly edematous cells with mild acantholysis | GM |
| | Papillary dermis | GM | PN + AC | GM | GM |
| | Reticular dermis | GM | PN + AC | GM | GM |
| Total time after exposure | | 4 h | 4 h | 4 h + 15 = 4 h 15 | 4 h + 10 = 4 h 10 |
| **24 h** | Epidermis | GM | Total necrosis | Very edematous cells with a very clear cytoplasm | GM |
| | Papillary dermis | GM | PN + AC | PN + AC | GM |
| | Reticular dermis | GM | PN + AC | Lesser alterations | GM |
| Total time after exposure | | 24 h | 24 h | 24 h + 15 = 24 h 15 | 24 h + 10 = 24 h 10 |

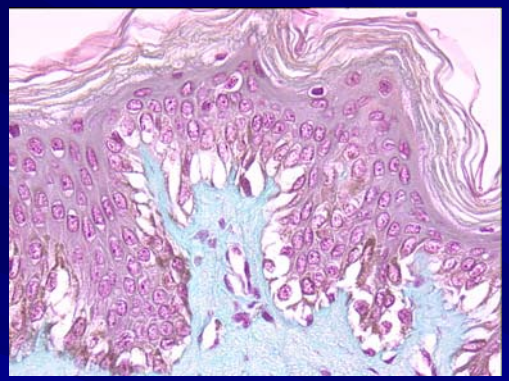
# Morphology at 24h

**Without washing**



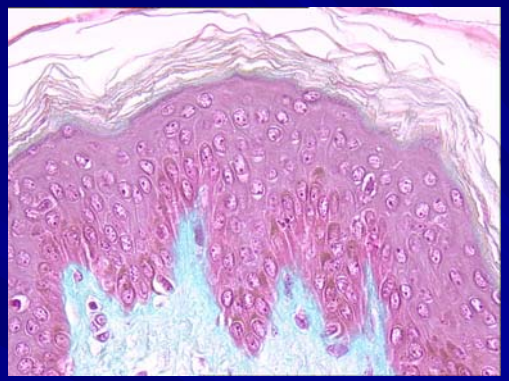
**Total necrosis with grey cytoplasm, pyknotic nuclei and acidophilic cytoplasm in papillary and reticular dermis**

**Water + Ca Glu**



**Many edematous cells in basal epidermis with very clear cytoplasm and basal membrane disruption. Pyknotic nuclei and acidophilic cytoplasm in papillary dermis; same lesion but weaker in reticular dermis**

**Hexafluorine®**



**Normal morphology in all layers**

# Human Skin Explants *ex vivo* *Conclusions*

- In the experimental condition utilized, 70% HF penetrated in < 5 minutes and caused massive damage to various skin layers
- After 20 seconds of 70% HF contact, Tap Water washing + Topical Calcium Gluconate Gel allowed temporary slowing of HF action on tissues, but a new evolution of injury occurred after 24 hours

# Human Skin Explants *ex vivo* *Conclusions*

- Washing with 200 mL of Hexafluorine® over 5 minutes associated with better results at 5 minutes and 1 & 4 hours
- With an ***absence*** of HF injury at 4 & 24 hours
- Washing with 200 mL x 2 of Hexafluorine® (400 mL total) over 5 minutes was associated with ***no tissue injury*** after 5 minutes to 24 hours

# Human Skin Explants *ex vivo* *Conclusions*

- Hexafluorine® should be considered as the initial decontamination measure for HF skin splashes