Comparative Experimental Decontamination of concentrated hydrofluoric acid (HF) in an *ex vivo* human skin model

Mathieu L[1], Lati E[2], Burgher F[1], Gasser P[2], Hall AH[3], Peno-Mazzarino L[2], Maibach HI[4], Blomet J[1]

[1]PREVOR Laboratory, Valmondois, France
[2]Center of Biological Research and Cutaneous Experiments, Clamart, France
[3]TCMTS, Laramie, Wyoming, USA and Department of Preventive Medicine and Biometrics, University of Colorado Health Sciences Center, Denver, Colorado, USA
[4]Department of Dermatology, University of California-San Francisco, San Francisco, CA, USA

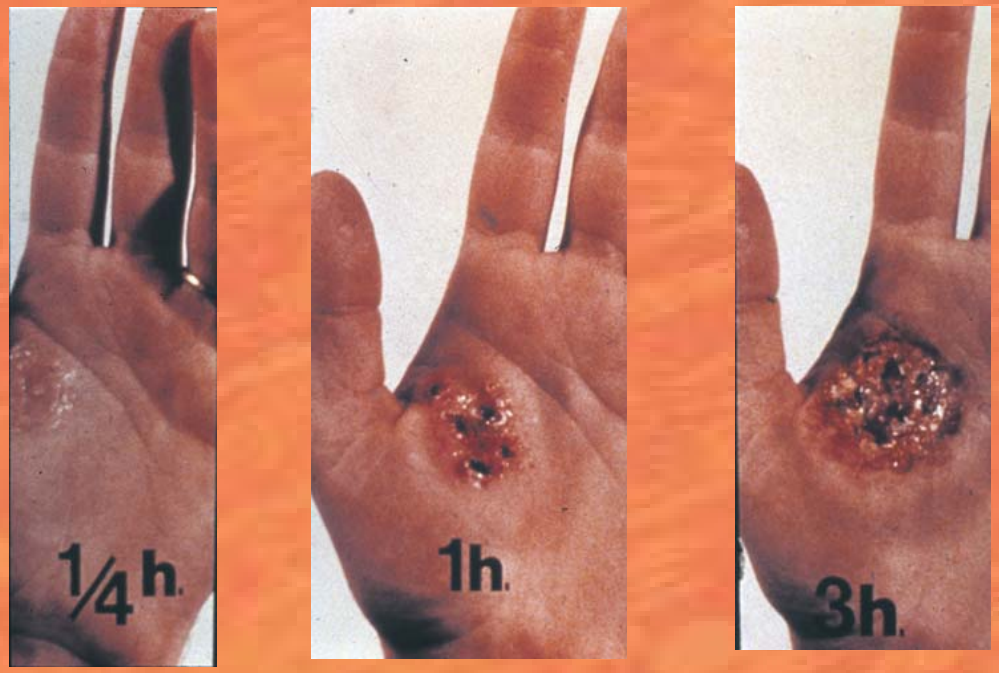
HF burns: a double aggressiveness

HF burns are due to

- its corrosivity (H^+ proton) +

- its toxicity (F^- fluoride ions) with a lethal risk

The HF burns depend on the concentration and the surface of the contaminated area.



H^+Cl^-

Hydrochloric acid

Na^+Cl^-

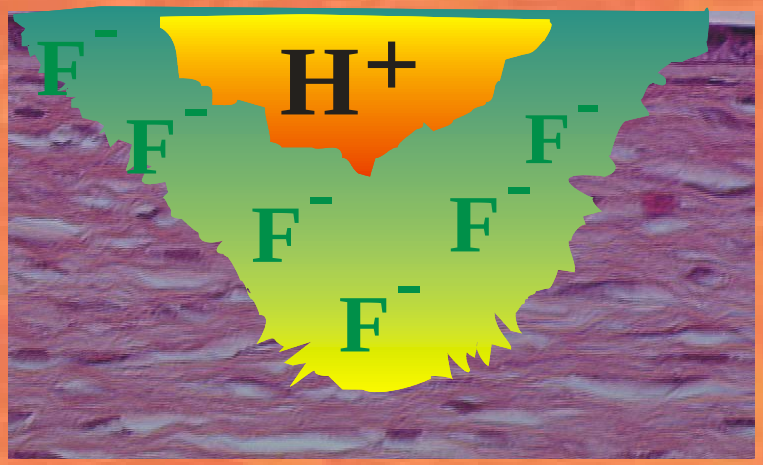
sodium chloride

Na^+F^-

Sodium fluoride

H F

hydrofluoric acid



Corrosion
qand necrosis
by chelation of intracellular calcium

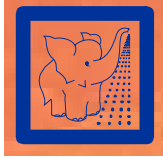
Emergency care of HF splash

✉ Washing with water

- ✓ Mechanical effect at the surface (removal and dilution)
- ✓ Followed by treatment on the skin
  - ✓ **calcium gluconate**
    - ✓ Also with DMSO
  - ✓ Zephiran salts (benzalkonium chloride) or
  - ✓ Hyamine salts (benzethonium chloride)
    - ✓ application with gauzes in ice
  - ✓ Iodine formulation
    - ✓ inhibition of apoptosis and proteinase activity
- ✓ On the eye
  - ✓ Only water
  - ✓ Or 1% calcium gluconate solution

✉ Washing with Hexafluorine®

- ✉ Specific emergency rinsing solution of HF splashes
- ✉ To be used **both on skin and eye**
- ✉ Mechanical effect at the surface as washing with water
  + neutralising acid, chelating fluoride ions and hypertonic
- ✉ Safe solution (**Non irritant** for eye/skin, **Non toxic** (LD_{50} > 2000 mg/kg), **Non sensitising)**
- ✉ Hexafluorine® can be followed by calcium gluconate treatment in case of delayed use



Hexafluorine ® efficacy for human eye/skin HF splashes

- **Results in occupational medicine in Europe**

  – **32 cases with exposure to 5-70% hydrofluoric acid**

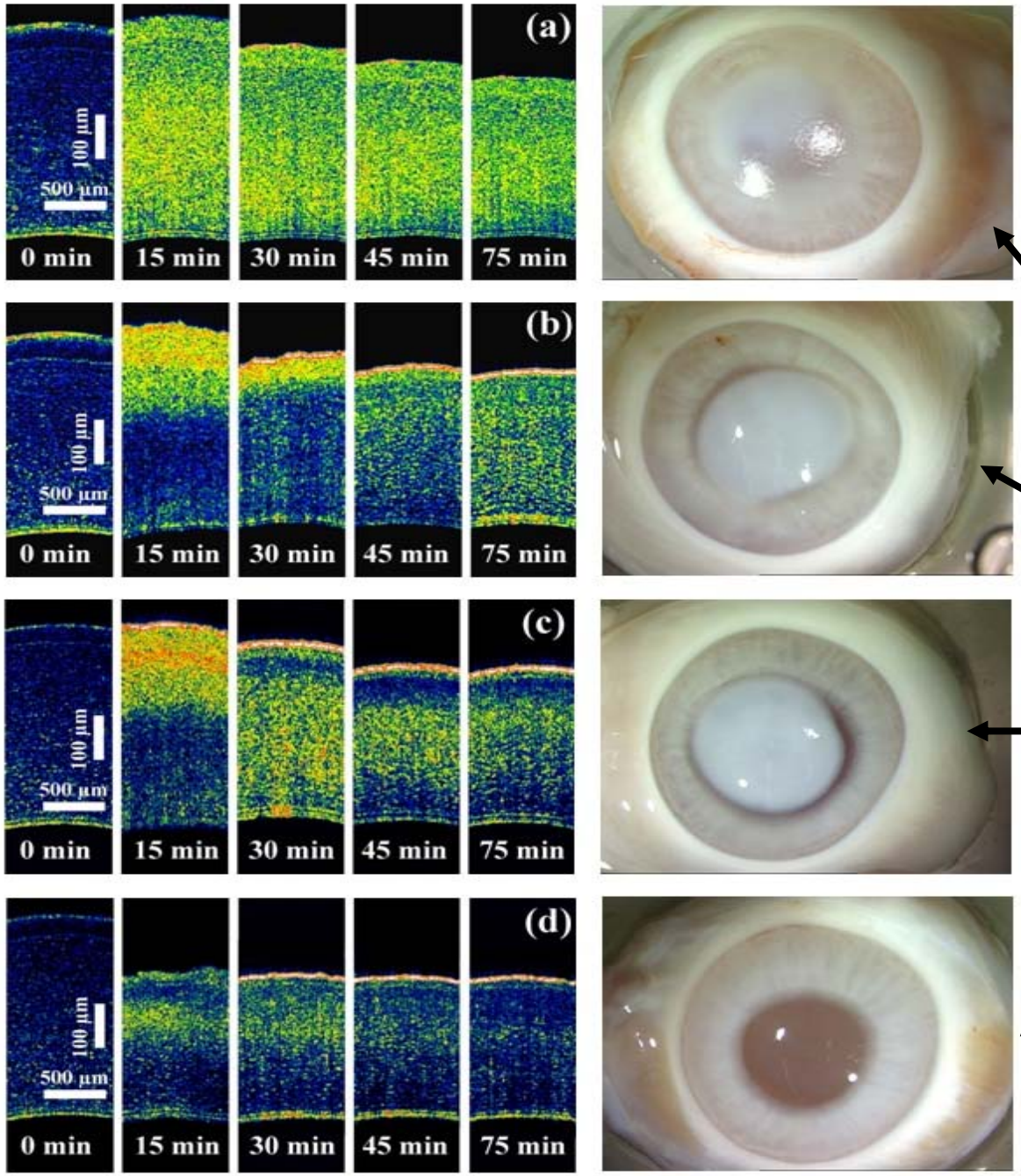
  5 of the 32 cases could have presented a lethal risk

  - **Decrease of the pain sensation** after the washing with Hexafluorine®
  - **No systemic effects,**
  - **Decrease or lack of sequelae**,
  - **Decrease** of the need of **secondary care**,
  - **Decrease** of **loss of work**

Hall AH et al, SSA J, 2000, 14, 30-33

Mathieu L et al, Vet Hum toxicol 2001, 43(5), 263-265

Soderberg K et al, Vet Hum toxicol 2004, 46(4), 216-218



Rinsing after 20 sec with 25 µl of 2.5 % HF with different solutions:

no rinsing,

rinsing with tap water for 15 min,

rinsing with 2% calcium gluconate for 15 min,

rinsing with Hexafluorine® for 15 min.

Clear cornea = no burn

Spoler F and coll. Burns 2008, 34(4), 549-55

Human skin explant model

The objectives of the study are:

- to highlight the extent of epidermal and dermal lesions following contact with concentrated hydrofluoric acid on living explants of human skin,

- and to compare the efficacy of different decontamination solutions.

The study was performed at Bio-EC laboratory (Clamart, France).

Observation allows characterization of the nature and extent of the lesions of the tissues and of the cells.

Materials and methods

•Preparation of Explants

86 explants, with a diameter of approximately 10 mm, were prepared from abdominoplasty from a 35-year-old woman (Ref P556).

Two explants have been used for each measure.

The explants were preserved alive in BEM medium (BIO-ECs Explant Medium) at 37° C in a moist atmosphere with 5% CO_2.

• Exposure was made with 70% hydrofluoric acid (FLUKA Ref. 47610, Lot 7125A, exact concentration = 73.0%)

•Two washing methods

- Running water and a single application of calcium gluconate gel (KAYS MEDICAL, Lot F02 2011/09 2.5% gel)

- Hexafluorine® spray (Mini-DAP 200 ml, Lot F971201C, expiry date: 04/03/2009)

Group	Number of explants	Treatment	Sampling Time
Control (untreated) group (T)	20	None	0, 20s, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 24 h
Hydrofluoric acid group (F)	18	70% HF	20s, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 24 h
Group Hydrofluoric acid + water washing + calcium gluconate gel (FPW)	16	70% HF 20s exposure then rinsing with water + Calcium gluconate gel	After the end of washing after 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h
Group Hydrofluoric acid + Hexafluorine® spray (FPH)	16	70% HF 20s exposure then rinsing with Hexafluorine®	After the end of washing after: 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, and 24 h

Materials and methods (2)

Histology

The samples were dehydrated, impregnated in paraffin and sliced **after 48 hours** of fixation in ordinary Bouin's solution.

General morphology was carried out by optical microscopy (X40) after Masson's trichrome (Goldner variant) dying.

Alterations were searched for in the **epidermis,** papillary and reticular dermis.

Application of HF and washing solutions

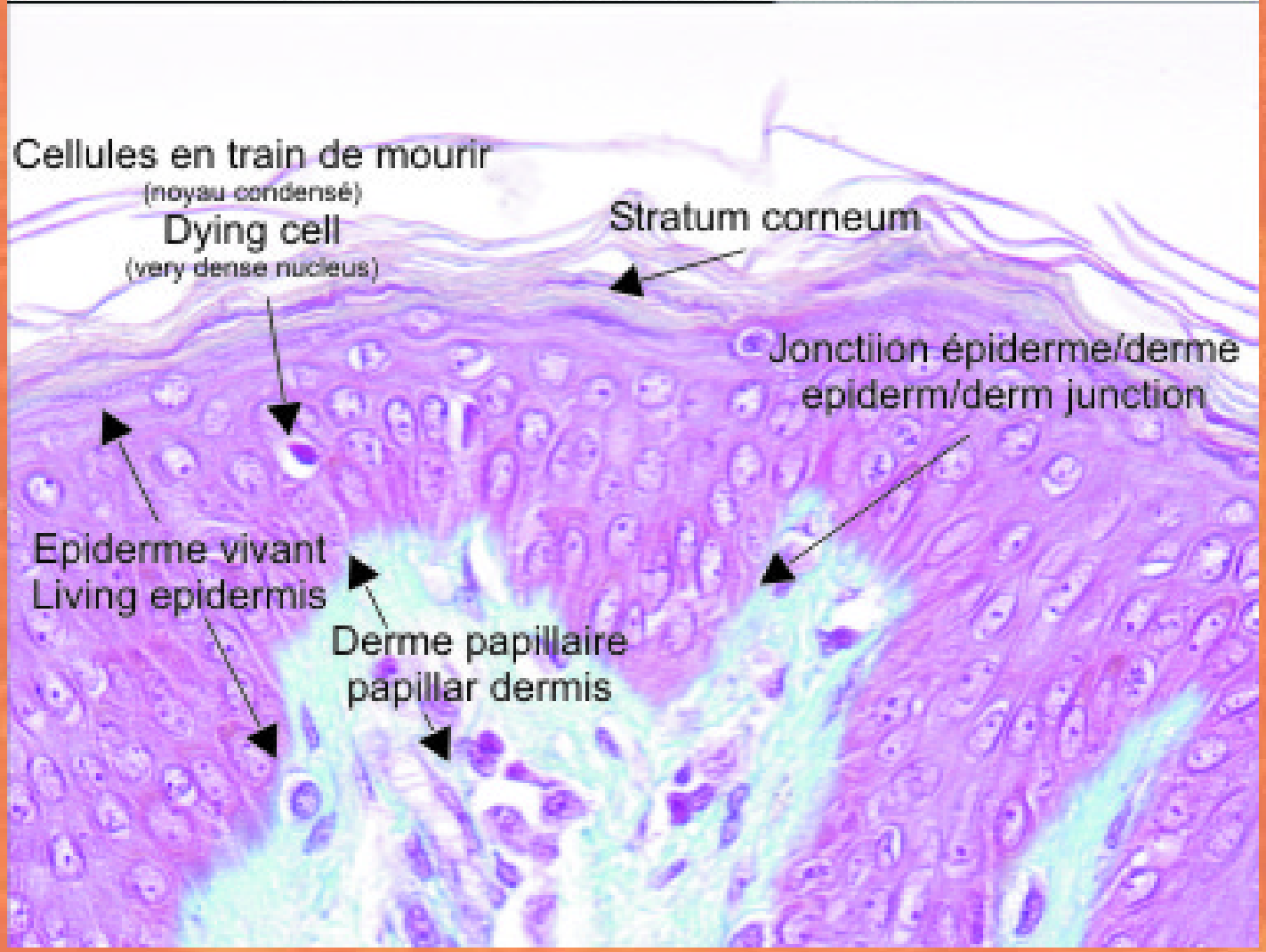
The hydrofluoric acid solution was applied by topical route to the explants by depositing filter paper disks previously saturated with 30 µl of 70% HF.

After 20 seconds of contact, the disks were removed and a group of explants were **washed with running water** for 15 minutes (approximately 2,000 ml per explant), followed by a single calcium gluconate gel application at a dose of 1g/cm^2.

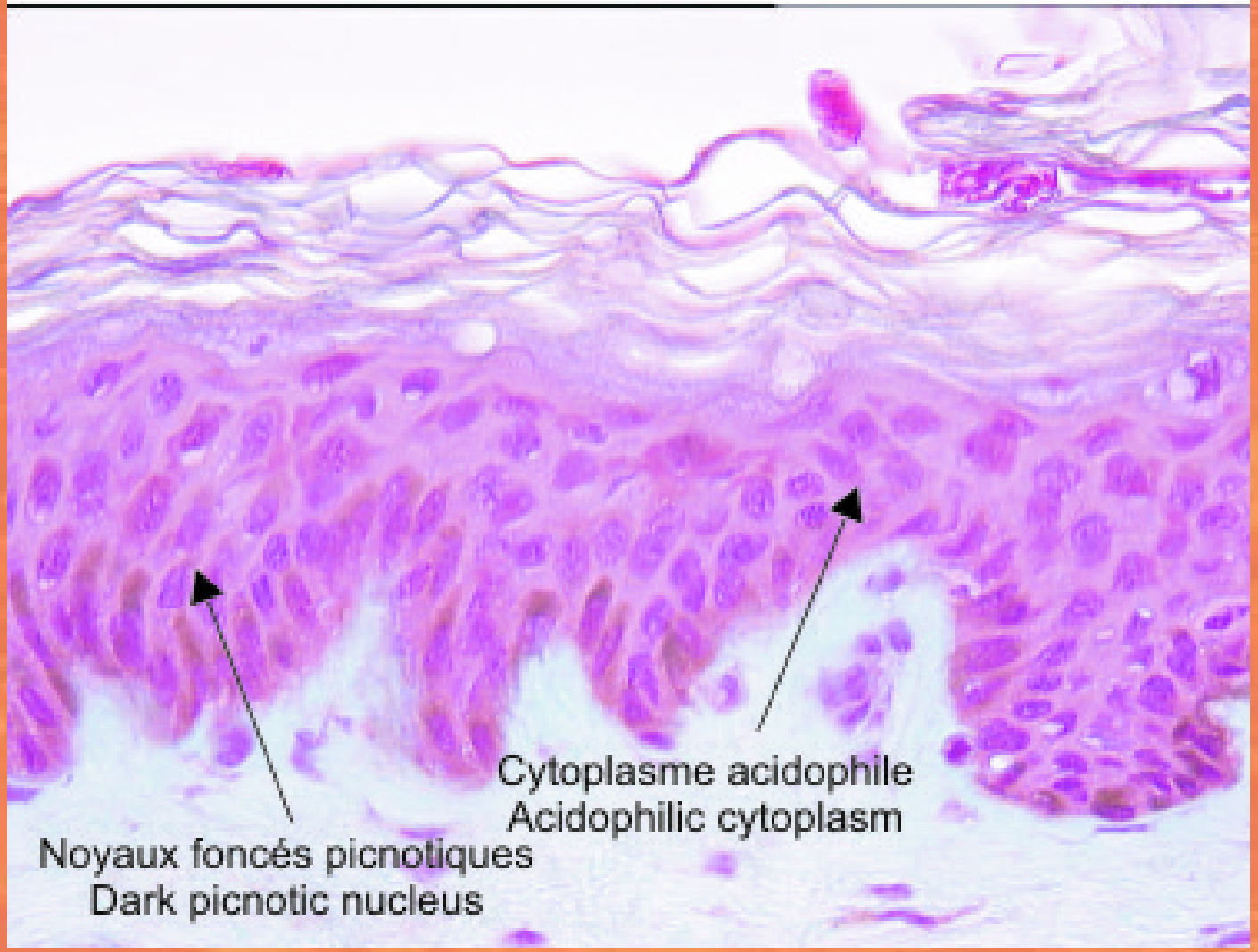
After 20 seconds of contact, the disks were removed and a group of explants were **washed with Hexafluorine® spray** for 400 ml).

The control group did not receive any treatment.

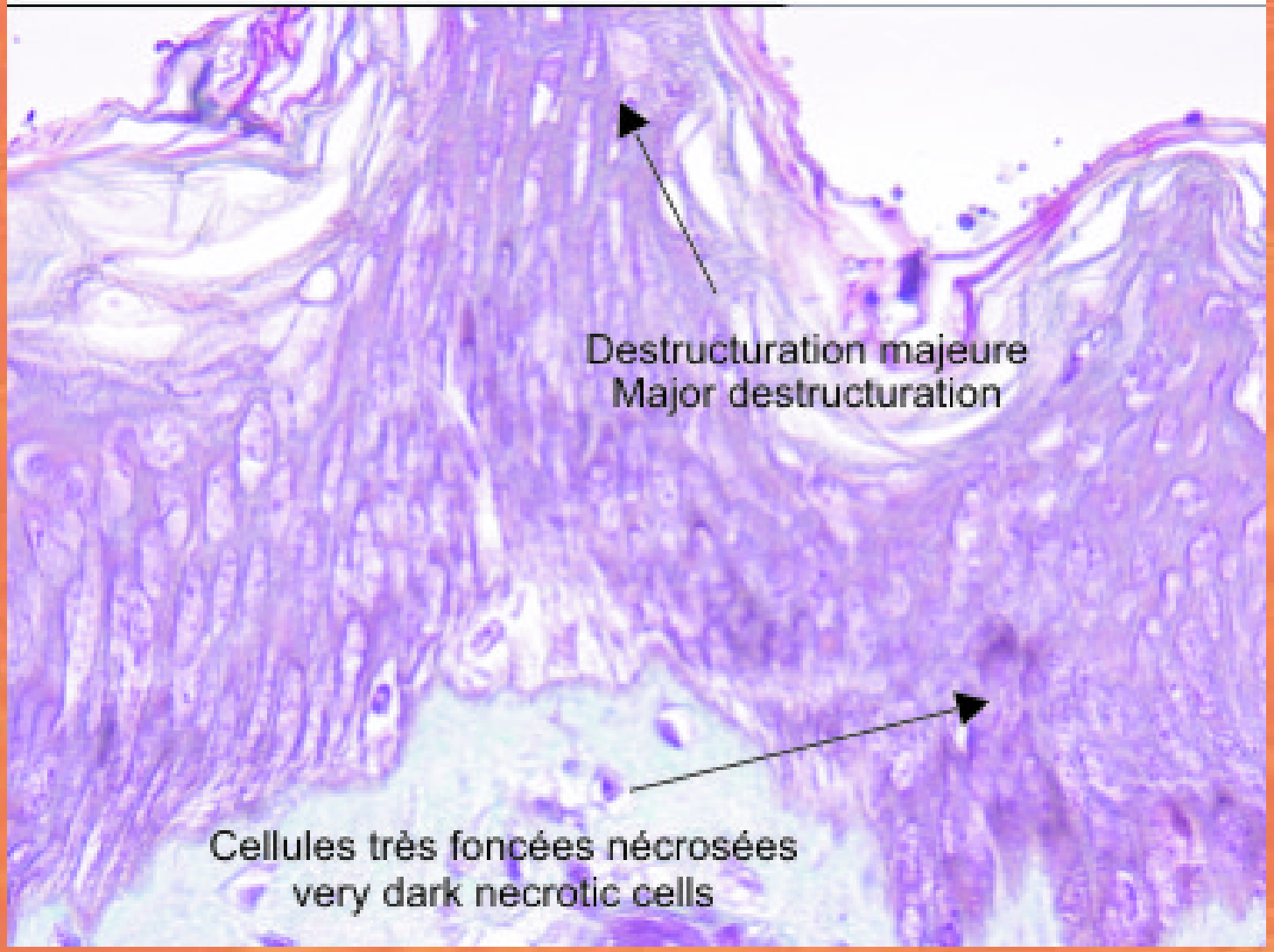
EXPLANT NON EXPOSE MORPHOLOGIE GENERALE
Non exposed explant general aspect



EPIDERME HF 5 MIN DE CONTACT
EPIDERMIS 5 MIN HF CONTACT

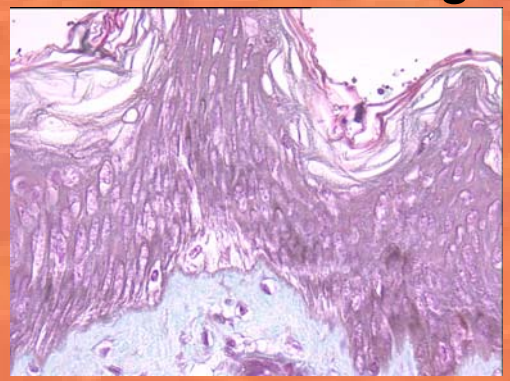
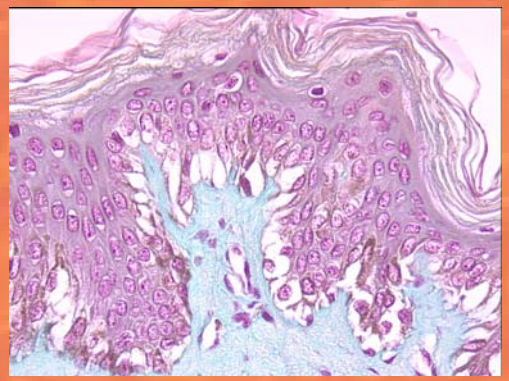
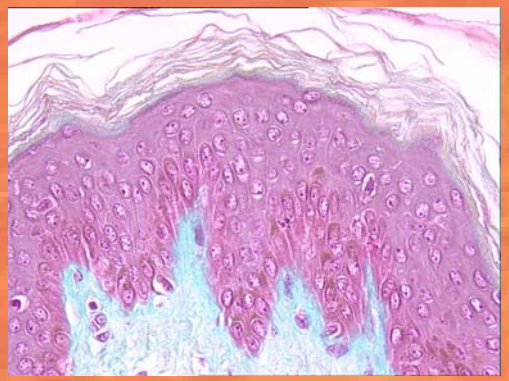


HF 24 H DE CONTACT NECROSE COMPLETE
HF 24 H CONTACT TOTAL NECROSIS



		Control group No exposure non washing (20 explants)	HF without washing (18 explants)	HF Water washing + Calcium Gluconate (16 explants)	HF Hexafluorine® washing 400 ml (16 explants)
T0	Epidermis	GM = 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	Water + Ca Glu	Hexafluorine®
		
Total necrosis with grey cytoplasm, piknotic nuclei and acidophilic cytoplasm in papillary and reticular dermis	A lot of edematous cells in basal epidermis with very clear cytoplasm and basal membrane disruption. Piknotic nuclei and acidophilic cytoplasm in papillary dermis; same lesion but weaker in reticular dermis	Normal morphology in all layers

Conclusion 1

In these experimental conditions, 70% hydrofluoric acid penetrated in less than 5 minutes and caused massive damage to various skin layers.

After 20 seconds of contact with hydrofluoric acid, water washing followed by a single topical application of calcium gluconate gel delayed the action of hydrofluoric acid on the tissues (10-15 min), and then the appearance of the burn. There are still some lesions after 24 hours which will justify, in practice, as it is well known, a repeated application of calcium gluconate gel.

The best washing results were observed in these experimental conditions with Hexafluorine® spray where no lesion appeared at all.

Conclusion 2

With the human explants model, there is no more need of extrapolation from animal to human. Furthermore, this type of test is in agreement with the new European REACH regulation.

This model will allow us to test other acids (such as sulphuric and nitric) and different bases (such as sodium hydroxide).

So, we will be able, in the very near future, to assess:

- the real time of penetration of HF (< 5 min),
- limits of chemical burns with point of no return,
- the maximal time of intervention for active decontamination,
- the inflammatory markers during the development of the burn lesions,
- even the healing process during the first week of evolution.

Thank you