Key Parameters of Hydrofluoric Acid Skin Contamination and First Aid Measures: Human Occupational Accidents and Experimental Data

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ABSTRACT

Background and Objective: Hydrofluoric acid (HF) induce severe skin necrosis through corrosive damage and tissue toxicity. As it is only partially dissociated (pKa 3.2), HF penetrates deeply into tissues. When absorbed into the circulatory system, HF can dissociate into H^+ and F^- ions (which binds calcium and magnesium and releases potassium), which together with resultant severe metabolic acidosis, may result in severe cardiovascular system toxicity with dysrhythmias and death.
Methods: A review of human occupational accidental HF exposures and recent experimental data from human skin *ex-vivo* implants was performed.
Results: Lesions due to 70 % HF appeared within the first minute on human skin explants and full skin penetration was observed within 5 minutes. Human accidental occupational exposure has occurred with 70% HF, 40% HF, and 6-15% HF/Nitric acid ("pickling acid") and Hexafluorine® has been found to be efficacious in these circumstances. Even with delayed use of this specific decontamination solution in 70% HF burns, the results compared with historical cases has been promising for preventing serious burns and systemic toxicity or mitigating them. A more recent experimental technique, using human skin explants obtained from abdominoplasty patients preserved *ex vivo* offers a method of evaluating the effects of corrosive substances and their mitigation with active decontamination solutions versus water irrigation in intact human skin. Hexafluorine used in this *ex vivo* model was more efficacious than tap water irrigation followed by calcium gluconate ointment. A validation study simulating a two- hand surface area confirmed Hexafluorine efficacy.
Conclusion: Decontamination of skin hydrofluoric exposure should be started within the first minute. Based on clinical and new experimental results, Hexafluorine decontamination seems to be a better alternative to usual tap water decontamination. In every case, calcium gluconate should be applied if it is required by medical protocol or if the burn has already appeared.

INTRODUCTION

Hydrofluoric acid (HF) is a relatively weak acid, but nevertheless is very toxic (Segal, 2000; Mackinnon, 1988). Industrial usage is widespread in metallurgy, organic chemistry, in the paper and semiconductor industries, and in analytical chemistry. HF is utilized in glassmaking for engraving (etching) and polishing. Its clinical manifestations were first described at the beginning of the 19^{th} century (Thenard, 1809; Chick and Borah, 1990). HF is manufactured from calcium fluoride (fluorospar, a clay-like substance) by reaction with sulfuric acid (H_2SO_4) (MacKinnon, 1988).

HF is a high-volume industrial chemical used in at least 8 different industries: electroplating, etching, flotation agents, integrated iron and steel manufacturing, laboratory chemicals, oil refineries, refrigeration, and semiconductors (Segal, 2000). HF production volume was 375,000 tons in 1998 and the U.S. 2002 demand was estimated at 400,000 tons (Segal, 2000). The U.S. HF demand was approximately 386,000 tons in 2001, and 2005 demand was estimated to be approximately 401,000 tons (CMR, 2002).

Serious HF systemic poisoning may occur in addition to dermal and ocular local effects (Segal 2000; Caravati, 1988). The corrosive action of the hydrogen ion (H^+) allows the fluoride ion (F^-) to penetrate deeply into the tissues and be absorbed systemically to bind calcium (Ca^{+2}) and magnesium (Mg^{+2}) ions and perturb the physiological electrolyte equilibrium, resulting in cardiovascular shock, severe lactic acidosis, cardiac dysrhythmias, and cardiac arrest (el Saadi et al, 1989; Chataigner et al, 1992). In concentrations of 50% or greater, HF causes nearly immediate skin burns associated with severe pain (Segal, 2000). Concentrations of 70% HF or the anhydrous form (>99%) are particularly dangerous (MacKinnon, 1988).

Recommendations for decontamination of HF splashes include initial water washing followed by topical application of calcium gluconate gel, magnesium oxide paste, or iced benzalkonium chloride. Hexafluorine® is an *active* hypertonic, amphoteric, slightly hypertonic, chelating agent specifically designed for initial decontamination of HF skin or eye splashes and may be considered as an alternative decontamination solution, compared to water washing, as it binds both the H^+ and F^- ions, thus preventing or inhibiting the acid tissue injury from the H^+ ion which allows deep penetration of the fluoride ion (F^-), responsible for local tissue injury and for life-threatening systemic effects.

METHODS

A review of human occupational accidental HF exposures and recent experimental data from human skin *ex-vivo* implants was performed.

RESULTS

Eye Decontamination: Hexafluorine®. Brief Summary: Hexafluorine® is a derivative of Diphoterine® with the molecular structure modified to include a specific fluoride ion (F^-) binding site. Its physical-chemical characteristics are similar to those of Diphoterine®. It has been shown to be an efficacious skin/eye decontamination solution for hydrofluoric acid (HF) workplace splashes in case reports and small case series.[7-9] **EVEIT Test**: An acute Ex Vivo Eye Irritation Test using optical coherence tomography (OCT) has recently been developed in Germany. Using rabbit corneas obtained from an abattoir and kept viable, a sturdy was done of corneal HF injuries and the effect of various decontamination measures. This method permits a dynamic analysis of HF penetration into the cornea and an analysis of corneal thickness changes following various interventions. After application of 25 µL of 2.5% HF, corneas were either left untreated or were decontaminated beginning 20 seconds after HF exposure. The decontamination solutions were: tap water, 1% calcium gluconate, and Hexafluorine®. Corneas left untreated developed complete opacification and OCT showed significant HF penetration. With tap water, there was also complete corneal opacification, but HF penetration was less as shown by OCT. Nearly complete corneal opacification was seen following calcium gluconate decontamination, while the HF penetration seemed somewhat less that with water. With Hexafluorine® decontamination, the corneas remained non-opacified (clear) and the penetration as shown by OCT was clearly less that with either water or calcium gluconate. Corneal thickness was evaluated in HF-burned and non-burned corneas following the different interventions. HF exposure caused corneal dehydration with or without decontamination. Significant swelling was seen following water decontamination, which resolved over 60 minutes. Calcium gluconate had a negligible effect on corneal thickness, while Hexafluorine® showed about 40% corneal shrinkage. Hexafluorine®'s hypertonicity is effective in causing corneal shrinkage and its efficacy as a decontamination solution is shown in that the HF exposed cornea remains clear for 75 minutes.

Human Case Reports/Case Series

A 70% HF facial vapor exposure occurred in a French industrial facility that manufacturers crystal and glass. A 35-year-old male technician was exposed to 70% HF vapor on the right cheek (approximately 1-2% total body surface area; TBSA) when opening a valve in the hydrofluoric acid circuit. Pain in the exposed area was immediate. Safety goggles were worn appropriately, so no eye exposure occurred. The worker immediately decontaminated himself with a Hexafluorine 5-Liter high-volume/low pressure portable shower, which resulted in rapid pain relief.

On medical examination in the facility infirmary, there were no clinical findings other than mild, painless erythema of the exposed area. The following day, erythema had essentially resolved and the worker had no pain sensation. Topical treatment with 3% calcium gluconate was initiated the day following the exposure as some painless erythema was still present. During the following week and at one month post-exposure, the worker was re-examined in the facility infirmary and no sequelae were noted. There was no lost work time (Siéwé et al, in preparation for publication, 2009).

70% HF exposure has occurred in Sweden with or without Hexafluorine decontamination (Soderberg et al, 2004). With Hexafluorine® decontamination, 2 workers with 70% HF exposure on a forearm or in the oral cavity had few and minor effects, while a worker with 70% HF exposure treated with the traditional water decontamination and topical and subcutaneous/intravenous calcium compounds suffered severe burns, systemic toxicity, and had about 1 year of lost work time (Soderberg et al, 2004). An additional 12/16 cases of HF exposure (some with 6% HF/15% HF/HNO_3) occurred in the same Swedish facility, and Hexafluorine® decontamination was associated with little lost worktime and no significant burns/systemic toxicity (Soderberg et al, 2004).

When only usual water decontamination and various treatments with calcium compounds were used, a worker accidentally exposed to about 85% HF when a valve failed did not have such a good outcome (Segal, 2008). Death has occurred is a similar case with 70% HF dermal exposure (Segal, 2000).

Amongst workers in a German mettalurgy facility with skin exposure to either 40% HF or "pickling acid" (6% HF/15% HNO_3), there was no lost work time, no sequelae, and no treatment required other than initial decontamination with Hexafluorine® (Mathieu et al, 2001).

Other cases involving a worker who fell into a vat containing 1,505 L of water, 30 L of concentrated HCl, and 233 L of HF with immersion of the entire body and face and 4 other workers with HF exposure who developed only minor injuries after decontamination with Hexafluorine® have been reported (Hall et al, 2000;Mathieu et al, 2007).

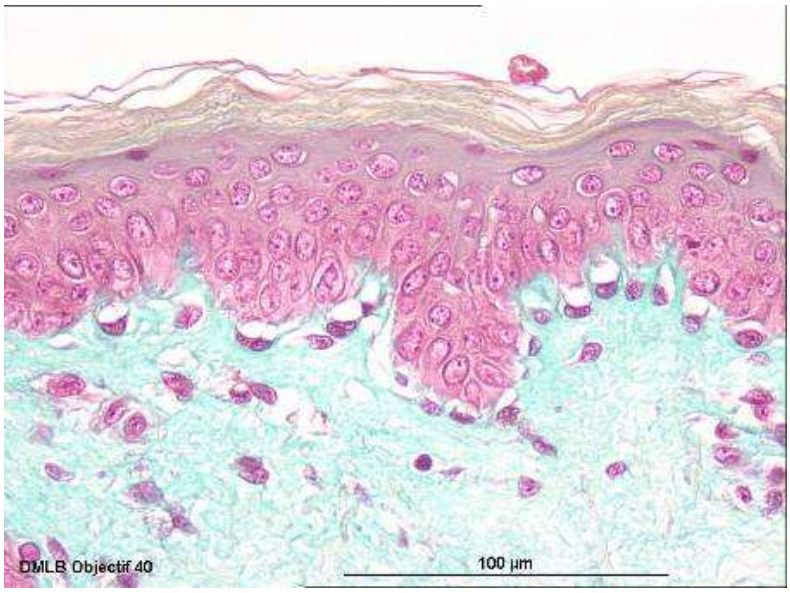
In general, workers with accidental HF exposure have had a better clinical outcome when Hexafluorine® was used as the initial decontamination solution as opposed to usual recommendations for potable water flushing.

70% HF on *ex vivo* Human Skin Explants

There is a lack of experimental studies which could objectively characterize the behavior of HF on human skin: mechanism of diffusion, kinetic of penetration through the skin and resulting cellular lesions. We propose here to describe the effects of 70 % HF using an *ex vivo* human skin model. The diffusion of 70 % HF starts within the first minute of contact at the surface of the epidermis. After 2 minutes, it reaches the epidermis basal layer. At 3 minutes, the epidermis is completely damaged and some lesions appear in the papillary dermis. Clear lesions of the papillary dermis are observed at 4 minutes. At 5 minutes, lesions appear in the upper part of the reticular dermis. 70 % HF needs only five minutes of contact to completely penetrate the human skin explants.

This experiment is reproducible and in accordance with both previous studies and clinical symptoms developing in case of accidental exposures
The study shows that the management of a chemical accident is a question of minute especially for the initial decontamination. Thus experimental method could be useful to objectively compare skin decontamination methods.

Introduction



Photos 2: Skin histological aspect after one minute exposure 30 µL of 70 % HF

- After 2 minutes of exposure, the skin presents 4/5 cellular layers with a definitely damaged morphology: cells with nucleus becoming pyknotic especially in the higher epidermis layers and cytoplasm becoming acidophilic (orange) in all the keratinocytes (photo 3). The cellular structures in the dermis present a good morphology.

HF is a particularly dangerous acid while it has a pK value of 3.2. HF is an acid which induces severe tissular necrosis due to a double mechanism of ions delivery:

- A corrosive hydrogen ion (H^+) associated with cutaneous[1], ocular[2] and respiratory[3,4,5] damages;
- A cytotoxic fluoride ion (F^-) responsible of local and systemic toxicity.

As HF is a small molecule not completely dissociated at the skin's surface, it can easily penetrate[6]. When the dissociation occurs, the skin is firstly altered by H^+. Then, the F^- liberated ion can develop, more progressively, its toxic properties due to calcium and magnesium[7] chelation.

$$HF \longrightarrow H^+ + F^-$$
$$Ca^{2+} + 2F^- \longrightarrow CaF_2$$
$$Mg^{2+} + 2F^- \longrightarrow MgF_2$$

Figure 1: Calcium, magnesium and fluor chemical reactions

The chelation of calcium and magnesium induces metabolic disorders[8,9] which lead to a delayed cellular death and a secondary tissular necrosis. Systemic[10] damages are potentially lethal[11,12,13,14] depending on the available amount of free fluoride ions.

Since the beginning of the 50's[15,16] the management of HF chemical burns has been considerably improved. Experimental studies were conducted to understand the evolution of HF burns and improve their decontamination. The need of a reliable model was studied, especially for skin contamination. Animal studies were performed on pig[17], rat[18,19] and guinea pig[20] but no reproducible model was found especially for high HF concentrations[21].

There is a lack of scientific experimental studies which could objectively characterize duration and mode of action of concentrated HF on human skin[22] via:

- The knowledge of diffusion's mechanisms and kinetic of penetration through the skin,
- The direct observation of the induced cellular lesions.

We propose here to describe an *ex vivo* human skin model burnt by 70 % HF. We chose this high concentration because it is one of the most important to be found in laboratories and widely used in industry. It is well representative of the most dangerous accidental occurrence in cases of splash because of the severity of burns and potential systemic effects.
The results of the experiment show the induced deteriorations and the progressiveness of HF burn from the surface of the epidermis down to the depth of the dermis.

Material and methods

The study was performed at BIO-EC laboratory France.
The tested chemical substance was: 70 % HF (FLUKA Ref. 47610, Lot 7125A, exactly titrated concentration of 73.0 %).
21 human skin explants were used and prepared by abdominoplasty from a 35-year-old woman (P556). The diameter of each explant was approximately 10 mm. The explants were preserved alive in BEM medium (BIO-ECs Explant Medium batch 060208) at 37° C in a moist atmosphere with 5 % CO_2.
The HF acid solution was applied by the topical route. We depose on each explant a filter paper disk (Medias Filtrans Durieux S.A. reference N°268 9 mm diameter) previously saturated with 30 µl of 70 % HF solution.
The disks were removed respectively after 20s, 1, 2, 3, 4 and 5 minutes of contact.
The total time of observation spread between 20 seconds to 5 minutes.

Histology sampling

Sampling was immediate just after the end of the exposure for a series of explants (exposure to 20 seconds, 1, 2, 3, 4 and 5 minutes). The following table (Table n°1) gives the total number of explants used. It also details the duration of exposure for each explant and the precise time of histological sampling.

Group	Number of Explants	Duration of exposure	Histological Sampling Times
Control	3	None	T0
HF 70 %	3	20s	20 seconds
	3	1 minute	1 minute
	3	2 minutes	2 minutes
	3	3 minutes	3 minutes
	3	4 minutes	4 minutes
	3	**5 minutes**	**5 minutes**

Table 1 – Number of explants and duration of exposure

Histology

After 48 hours of fixation in ordinary Bouin's solution, the samples were dehydrated and impregnated in paraffin with a Leica 1020 automatic dehydrator. They were placed in blocks with Leica EG 1160 coating station. 5 µm slices were made with a Minot-type microtome, Leica 2125, and pressed onto superfrosted silaneized glass histology slides.

Microscopic observations were carried out by optical microscopy with a Leica type DLMB microscope with a 40X objective. Photo-micrographs were performed with a CCD Sony

DXC 390P camera and stored with Leica IM1000 data archiving software. The observations of general morphology were carried out on paraffin slices dyed with Masson's trichrome, Goldner variant.
Cellular structures alterations were searched for in the skin 4 main layers (superficial and basal epidermis, papillary and reticular dermis).

Results
All the samplings of the non exposed control group had a very good morphology during the experiment. Histological aspects of normal samples:

- The *stratum corneum* is more or less thick, moderately laminated, very slightly keratinized at the surface and some more at its base. The epidermis presents from 4 to 5 cellular layers**.**
- The relief of the dermo-epidermis junction is moderate.
- In the papillary dermis collagen presents average thickness fibers forming a low density network. The cellular structures have a normal morphology.
- In the lower reticular dermis, the cellular structures also have a good morphology (photo 1).

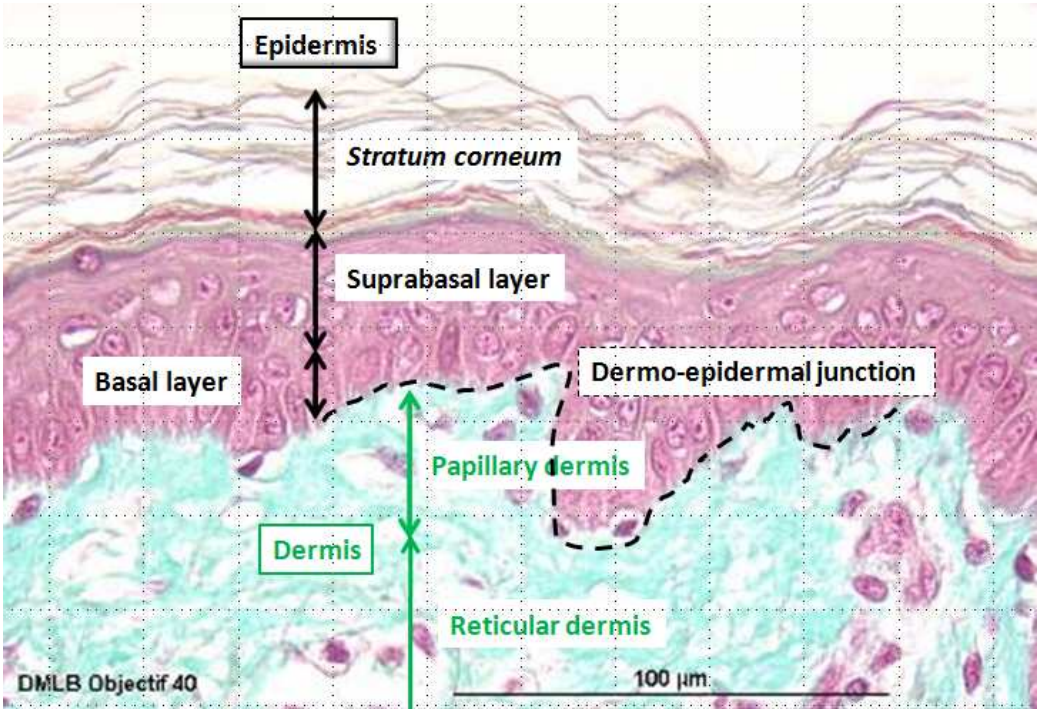


Photo 1: normal aspect of skin optical microscopy X40

Skin thickness measurements of the *ex vivo* human explant is specified in table 2:

Thickness (µm)			
Total skin	3972		
Epidermis	72	*Stratum corneum + granolosum + spinosum*	57
		Basal layer	15
Dermis	3900	Papillary dermis	248
		Reticular dermis	3652

Table 2: Thickness of the human skin explants layers

For the explants treated with HF 70 %, we observed:
- After a 20 seconds contact, no deterioration of the epidermal or dermal structures.

- After one minute of exposure, the epidermis presents 4/5 cellular layers with a slightly modification of morphology (cells with gray cytoplasm in the upper layer and nucleus becoming pyknotic). The cellular structures of the basal epidermis and all the dermis present a good morphology.

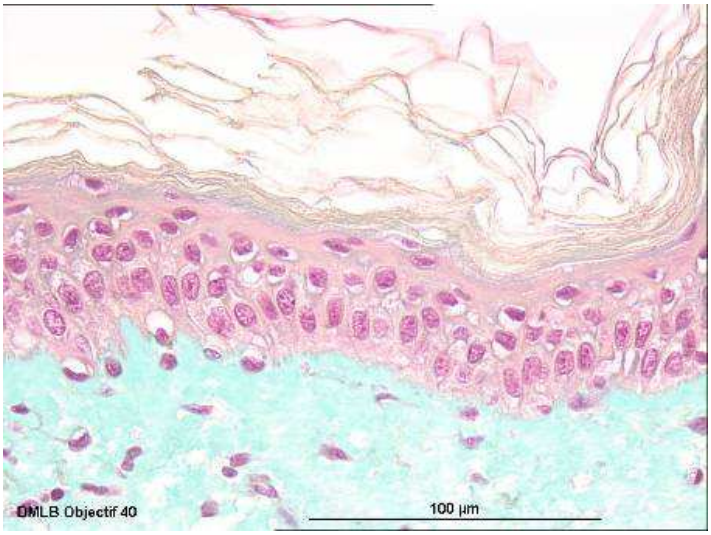


Photo 3: Skin histological aspect after 2 minutes exposure 30 µL of 70 % HF

- At the third minute of exposure, lesions of the 4/5 epidermis cellular layers are characterized by the presence of many cells with moderately pyknotic nucleus and edema surrounding the nuclei. At the base of the *stratum corneum* and in the basal epidermis layer as well, cells present characteristic cytoplasmic alterations with clear aspect. In the papillary dermis, the cellular structures are slightly pyknotic. The reticular dermis is still normal (photos 4).

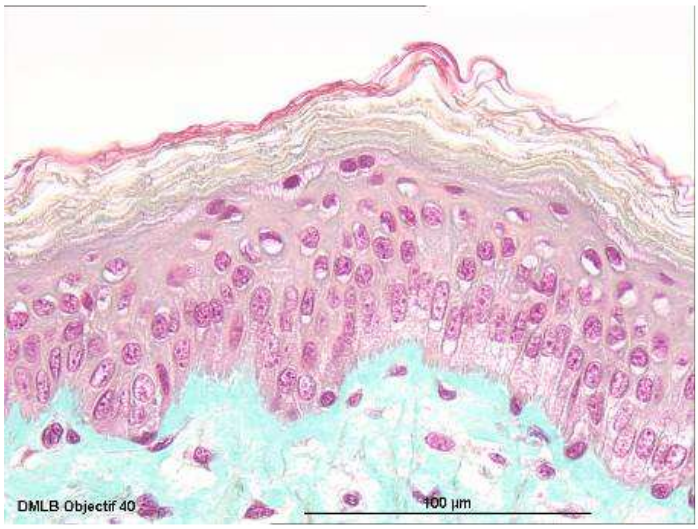


Photo 4: Skin histological aspect after 3 minutes exposure 30 µL of 70 % HF

- After 4 minutes of exposure, one notes exactly the same deteriorations in the epidermis as those observed at 3 minutes. In papillary dermis, cells present more clearly pyknotic nucleus. But there is always a normal aspect in the reticular dermis (photo 5).

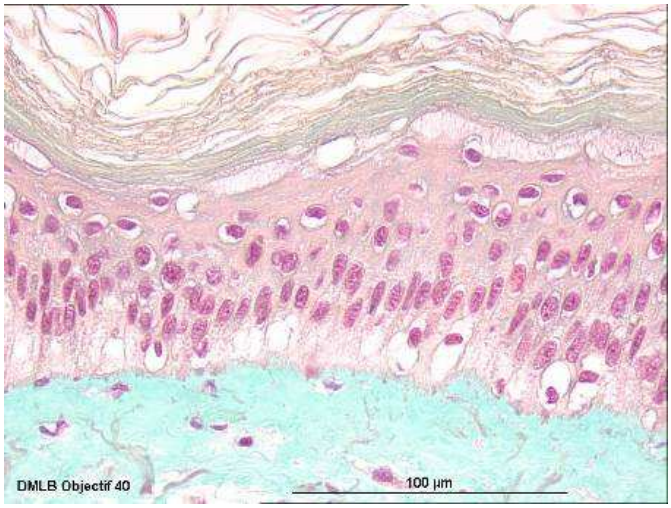


Photo 5: Skin histological aspect after 4 minutes exposure 30 µL of 70 % HF

- At 5 minutes of exposure, same lesions are observed in the epidermis and the papillary dermis as those observed at 4 minutes. But, moreover, HF reaches reticular dermis with apparition of slightly pyknotic nucleus cells in this deepest layer of the explants (photo 6).

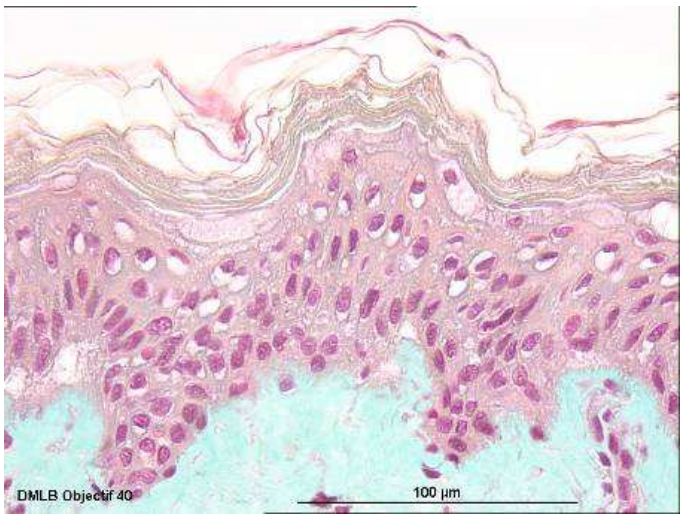


Photo 6: Skin histological aspect after 5 minutes exposure 30 µL of 70 % HF

Discussion

1 – Experiment's observations: reproducibility and details of histological lesions

In this study, we demonstrate that *ex vivo* human skin explants allow to observe in real time diffusion of 70 % HF through the epidermis and dermis as observed in animal studies and during accidental skin exposures.
Histological observations with human skin explants offer the possibility; after contact with the double destructive potential of concentrated HF; to follow very closely the reaction of tissue layer by layer in epidermis and dermis.
The human skin explants of the control group**,** maintained alive during all the time of the experiment, present for all the samples a normal cellular and tissular morphology.

The reproducibility of the experiment is attested by tests in triplicate with the observation of same results at any time. For the explants exposed du HF, the lesions are completely identical on the three series treated in parallel at all stage of exposure.

In our experimental conditions, we showed that 70 % HF does not make an immediate massive attack. The diffusion starts after 20s and within the first minute. The full skin penetration is complete in 5 minutes.
The beginning of the first epidermal lesions at the first minute is in accordance with both experimental data[23] and reports of previous accidental situations[24].
After 1 min of contact, light cellular deteriorations appear in the superficial epidermal layer.

They increase after 2 min of contact, with the presence of clearly acidophilic cytoplasm with typical pink-orange color. The lesions are stronger after 3 minutes of contact with the appearance of cells edema in the epidermis and slightly pyknotic cells in the papillary dermis. After 4 min of contact, the epidermal lesions are very strong with definitely pyknotic cells in the papillary dermis. After 5 min of contact the lesions are very clear in the epidermis and the papillary dermis. There are weak alterations in the lower reticular dermis.
Both tables (tables 3 and 4) hereafter summarize the data.

Table 3: Dynamic of penetration of 70 % HF after 20 seconds of skin exposure

Duration of exposure	Microscopic morphology
1 min	Beginning of penetration in the higher part of the epidermis
2 min	Attack of the basal deep layer of the epidermis
3 min	Epidermis completely damaged. First lesions of the papillary dermis (superficial part of the dermis)
4 min	Epidermis completely damaged. Clear attack of the papillary dermis.
5 min	Epidermis completely damaged. Beginning of attack of the reticular dermis (deeper layer of the dermis).

Table 4 gives the detail of cell's alterations layer by layer. Abbreviations: Good morphology (GM), Pyknotic Nucleus (PN) and Acidophilic Cytoplasm (AC)

20 s	Epidermis	GM (good morphology)
	Papillary dermis	
	Reticular dermis	
1 mn	Epidermis	PN + AC slightly (Pyknotic nucleus and acidophilic cytoplasm)
	Papillary dermis	GM
	Reticular dermis	
2 mn	Epidermis	PN + AC
	Papillary dermis	GM
	Reticular dermis	
3 mn	Epidermis	PN + AC
	Papillary dermis	PN + AC slightly
	Reticular dermis	BM
4 mn	Epidermis	PN + AC
	Papillary dermis	
	Reticular dermis	GM
5 min	Epidermis	PN + AC
	Papillary dermis	
	Reticular dermis	PN + AC Slightly

Table 4: Detail of histological cell's alteration during 5 minutes of skin exposure to 70 % HF

We observed cellular alterations but the tissular structure stay with a coherent aspect. The epidermis is damaged, but still present. For our part, under the precisely described

experimental conditions, limited to the 5 first minutes of contact, we did not observe any massive liquefactive necrosis (denaturation of the cell and disintegration of the structures which are not recognizable any more) while it is frequently described during the clinical following of accidental cutaneous chemical burns in man[xxv].
As described by Ohtani[xxvi] in its human case report with 60 % HF lethal burn, we also noted that the epidermis was still visible by microscopic observation. This effectively may be one of the unique pathological features of the skin lesions in cases of HF burns. These facts could be due to specific behavior of HF in opposition to others inorganic acids. We will discuss this question further on.
In practice, the experiment confirms that it exists an about one minute delay to carry out an effective washing.

2 – HF Chemical reactivity and penetration's mechanisms

The damages observed are due to chemical and physical properties of HF. This acid is considered as a particularly dangerous corrosive and protoplasmic poison while it is a small molecule (MW = 20) and a partially dissociated acid (pK = 3.2). Compared to a strong acid, such as hydrochloric acid, it is 1000 times less dissociated. So, HF is less reactive in surface than other strong mineral acids.
Authors[xxvii,xxviii] suggest that HF is non-dissociated at the surface of the skin and can penetrate easily through the epidermis. For these authors, it would be able to cross without difficulties the lipidic membranes. Matsuno in 1996[xxix] suggests also that undissolved hydrogen fluoride rapidly penetrates the skin. Gutknecht[xxx] studied HF transport through lipid bilayer membranes. He makes hypothesis that F^- transport through biological membranes occurs mainly by nonionic diffusion of HF. Membrane permeability's to HF range from 10^{-4} to 10^{-3} $cm.s^{-1}$, five to seven orders of magnitude higher than the permeability to F^- and H^+.
Dissociation with liberation of F^- ions will occur secondarily in deeper tissues. The liberated fluoride ion attacks enzymes and cell membranes[xxxi].
The formation of salts with tissue cations such as calcium or magnesium drives progressive dissociation of HF molecules. The residues are relatively insoluble and stable (pK_s CaF_2 = 10.5 and pK_s MgF_2 = 8.2), precipitating within the tissues[xxxii]. Other fluoride salts are much more soluble and dissociable[xxxiii], liberating the fluoride ion which remains available to chemically react with tissues[xxxiv].

Because it has a double mechanism of corrosivity (with H^+ ion) and toxicity (with F^- ion), HF first creates a corrosive burn at the surface of the epidermis. Secondary, it will completely disrupt the physiological equilibrium while chelating cellular calcium and magnesium ions causing secondary cellular necrosis.
The necrosis process and/or the potential to systemic diffusion are then strictly dependant of the amount of poison F^- ion near the blood vessels of the deeper skin layers.

Kirpatrick[xxxv] has underlined that the characteristic pain of HF burns is thought to result from the immobilization of calcium ions in the tissues, causing nerve stimulation by the shifting of potassium ions. In addition, hyperkalaemia can occur (excess of potassium liberated by the damaged cells) with risk of cardiac failure by ventricular fibrillation[xxxvi].

The kinetic of HF skin penetration is clinically known through the observed symptoms during accidental exposures. Based on analysis of accident in workers, high concentration HF burns are known to completely develop in the following minutes2[26]. To improve and to make more specific the immediate first care, one try to understand more precisely the initial lesions following HF contact. For these reasons, many experiments have been conducted in animals.

3 – Limits of animal's models

Using animal is always questioning upon relevance to human exposures. Animal models shows different kinetic of histological damages comparing with theses knew in human accidents. Possible explanation for non mimic to human in animal model could be the different penetration coefficient.
Animal tissues could have a greater diffusion for chemicals than in the human skin. Maibach HI[xxxvii,xxxviii] gives general data on animal models in dermatology, interspecies difference for skin absorption and rate of the penetration of toxics. He suggests that the differences could be due to differences in skin structure and chemical composition. Even in the pig model, which was often compared to the closest skin epithelial tissue to humans, the speed of HF penetration was found too fast even with a very short time of exposure[18].
Noonan[xxxix] has worked on different mammal's tissues and concentration. He was not able to establish a reproducible model: sometimes too fast full thickness skin penetration was observed or nothing, even on human post mortem explants. No correlation was possible between guinea pig model and human skin explant.
T. Noonan conclude about a guinea pig skin model that it was difficult if not impossible to create a consistently partial thickness necrosis injury in that animal skin using a wide variety of concentrations of HF and duration of exposure.
Sarika Saggar[xl] gives a lot of detail upon ethnic differences in skin properties with objectives data measurements. Other factor could depend on the skin localization (back, leg, ear…) or the various duration of exposure. But, the main factor could be the necessity to prepare the area to be burns before exposure by shaving or using depilatory creams. These preparations modify the quality and the structure of the epidermis and of the cutaneous appendices. The induced micro traumatisms could highly increase the animal skin susceptibility to HF injury.

Recently, the use of an *ex vivo* model of rabbit eye[xli] allowed to observe both diffusion, kinetic and damages due to 2.5 % HF solution on *ex vivo* enucleated rabbit cornea. HF needs only 4 minutes to penetrate the complete cornea. This model with weak concentration allows good observation.
In conclusion, the extrapolation from the animal model to the behavior of a human skin still poses methodological problems unsolved up to now.
Our results make it possible to avoid any approximate extrapolation from an animal model to a human skin.

4 – Human's skin:

- Layers depths variability
- Topographical variations
- Abdominal explants description

With human's skin explants, maintained alive during all the experiment duration, it becomes thus possible to observe concentrated HF burns directly. This is why we chose to work directly on human skin explants.

The skin presents two main parts: the more superficial, the epidermis and the deeper the dermis. The epidermis is a malpighien, stratified epithelium composed, near the surface, by the *stratum corneum* and more deeply by the *stratum granulosum* and *spinosum*. The deepest part is a single cell germinative layer: *stratum* basal (See photo 1).

The epidermis is supported by a subepidermal basement zone, a membrane that borders epidermis and underneath dermis.

The dermo-epidermis interface presents an undulating appearance, with intermittent regular protrusions of the epidermis layer (rete pegs) into the upper layers of the underlying dermis. The small area of epidermis between rete pegs is called the suprapapillary plate. It is possible to oppose the suprapapillary skin (thinness) to the interpapillary skin.

The dermis can be dived into the upper papillary, that is the thinner of the two, and the deeper reticularis layers. The papillarydermis extends irregularly upward as "pegs" which interdigitate with the rete ridges from the epidermis[xlii]. One currently regards the skin as a cellular plate depressed with his deep face by the digitations of the upper dermis.

Skin thickness varies considerably between different races and age-groups, between men and women, and according to different regions of the body surface[xliii,xliv]. Lee[xlv] compared 452 biopsies concerning 28 different body sites, of Korean men and women. The table 5 gives the differential published value between maximum and minimum thickness of the skin. In addition, the last line shows the comparative data of our *ex vivo* explants.

Authors	Epidermis (µm)	Dermis (µm)	Total thickness (µm)
Cowdry[49]	20/100		
Maximow[50]	70/140	1000/3000	Calculated*: 1070/3140
Southwood[51]	20/140	400/2500	Calculated*: 420/3900
Artz[47]	39/64	956/1911	Calculated*: 995/1975
Lee[45]	31/637	469/1942	Measured*: 521/1277
Our explants	72	3900	Measured*: 3972

**Differences between the calculated values (sum of the minimal and the maximal data) and the measured one because the thinnest epidermis was not necessarily associated with the thinnest dermis and vice versa.*

Table 5 comparison between published values of skin thickness

Table 6 shows the differences between Korean and Caucasian skin layers. In addition, the last line shows the comparative data of our *ex vivo* explants.

Abdomen thickness (µm)	Epidermis	Dermis	% E (E+D)
Caucasian	**41/40**	**1640/1492**	**2.4/2.6**
Korean	**69**	**1248**	**6.0**
Abdomen BIO-EC (female)	**72**	**3900** (Papillary dermis 248 Reticular dermis 3652)	**1.8**

Table 6: Comparison of thickness of abdomen skin between races (Caucasian values taken from Southwood[xlvi] and Artz et al.[xlvii]) *versus* Korean from Lee. Last line: our experimental data

A lot of authors[xlviii,xlix,l] point out that the thickness of the epidermis varies with age, sex, race, various degrees of nutrition and in different parts of the body. It depends also partly of the technique of measurement and/or tissues fixation. For the epidermis, the chief difference is in the horny layer. The basal cell layer remained quiet constant in all the cases.
The dermis comprised most of the thickness of the skin and seems to vary more considerably from one region to another. Relatively thin in youth, it reaches is maximum thickness between 40 and 50 years. Then it declines until the thickness in old age is similar to that in childhood[li].

All these data make it possible to set the value of our *ex vivo* samples and to interpret them in comparison with others experimental or clinical observations following HF accidental splashes.
The abdomen skin thickness, in the Korean series, is ranged in the high part of medium values like neck (anterior), dorsum of hand, lateral thigh, a little bit more than cheek, axilla, arm (front and back), forearm (front and back) or leg (anterior, lateral and back).
Analysis of Korean *versus* Caucasian values shows 14 to 27.6 % more thickness for the Caucasian persons. This difference is more due to the epidermis part (70 % more thickness) than to the dermis (only 16.5 % more thickness).
Sexual differences of thickness of epidermis, dermis and whole skin: the total value (epidermis + dermis) are 14.8 % more for males than for females. The differential for epidermis is much less important: 0.5 µM in average. The differential for epidermis is around 217 µm (16 % more for males).

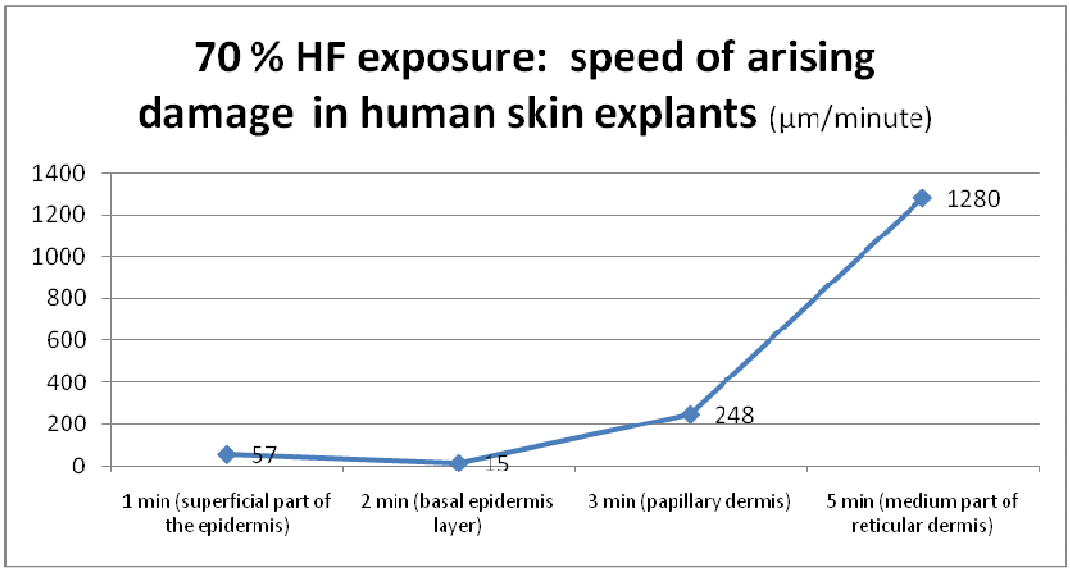
Probably because of the reason of surgical abdominoplasty, we observe a much thickness skin (epidermis + dermis) for all our samples. This thickness is mainly supported by dermis because our epidermis measure in average 72 µm, a quite similar value to those of the Korean abdominal epidermis (79.4 µm) . The female origin of the *ex vivo* explants could not be a problem comparing with male skin because there is quite no difference for the epidermis thickness in the Korean series.

For the following of the burn's evolution down from the surface to the depth, we observed essentially lesion of the epidermis layer and only of the upper part of the dermis. At the fifth minute of exposure, the lesions are clearly settled in the papillary dermis and just begin to reach the medium part of reticular dermis reaching 1500/1700 µm of depth. So we do not think that the difference of thickness, essentially due to the reticular dermis, make a problem for a dynamic interpretation of the burn progressiveness.

5 – Kinetics of penetration through human skin

The key points for the development and severity of HF cutaneous burns are: HF concentration, time of contact, percentage of body surface exposed and skin penetrability. But, there is lack of knowledge for tissular damages, regarding time needed for full skin penetration due to concentrated HF.
With the present experiment we can approximate the speed of penetration into each skin layer via the appearance of the layers lesions.

It is widely acknowledged that the rate limiting barrier to the absorption of most topically applied chemicals is the stratum corneum[lii]. The observed penetration speed through the *stratum corneum*, the *granulous* and *spinosous* layers is around 57 µm per minute.
Then, the speed slows to 10 µm per minute. It takes about one more minute to cross the lower basal layer of the epidermis. This slowing down may be due to the specific structure of the acellular dense connective tissue of the basement membrane separating the epidermis from the upper dermis.
After, the speed accelerates up to 248 µm per minute through the papillary dermis. At the end of the experiment (5 minutes), HF damages are as deep as 1600 µm from the skin surface.
In the reticular dermis, the speed reaches 1280 µm per minute probably due to the specific composition and density of this layer (graph 1).



Graph 1: Kinetics of 70 % HF through human *ex vivo* skin explants

Conclusion

Under our experimental conditions, the human skin explants burned by 70% HF is a reproducible experiment.

The first cellular deteriorations, due to 70 % HF, appears within the first minute. Full penetration is observed in 5 minutes.

The study confirms the severity and the speed of penetration of 70 % HF burns. The kinetics of observed cellular damages demonstrates that an initial management, in case of chemical accident, is a question of minute to prevent or to minimize the severity of the chemical burns due to concentrated HF acid.

Further experiments will be performed in the same experimental conditions to show comparative efficiency of various decontamination solutions.

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