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IN VITRO VISUALIZATION OF HYALURONIC ACID DERMAL FILLER INJECTION IN HUMAN SKIN: A NEW MODEL FOR DERMAL FILLERS STUDY

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CONFLICT OF INTEREST

- All the autours are full time employees of Galderma R&D

INTRODUCTION

- In recent decades, injectable dermal fillers are becoming very useful for the correction of congenital or traumatic facial defects and in patients suffering from lipodystrophy following AIDS. Moreover, these substances are becoming very popular for the treatment of facial wrinkle.

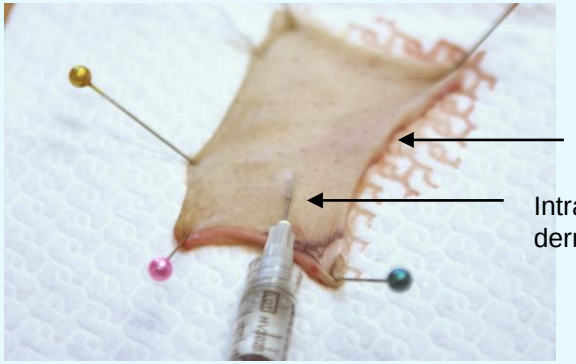
- There are numerous dermal fillers available based on different materials. Current nonclinical assessment of dermal filler performance mainly involves *in vitro* analyses, which are poorly indicative, or *in vivo* implantation studies in animal models which are time consuming.

- The aim of this work was to establish an *ex vivo* model using excised human skin as a valuable and rapid screening model providing information concerning the distribution and subsequent diffusion of the injected product in skin tissues using dermo-echography and histopathology evaluation techniques associated with image analysis.

METHODS

- Frozen human skin samples obtained after abdominal surgical procedures from three different donors were used after thawing.
- Three intradermal injection of Hyaluronic acid based dermal filler Emervel® Classic were performed on each skin sample according to an *in vitro* human skin dermal filler injection protocol developed at Galderma R&D to study the tissue distribution of dermal fillers .

- Dermal filler injection volume: 100 µL and 200 µL.



- After injection, skin samples were kept during 24 hours in organo-culture medium and kept at 37°C, 95% CO_2 and saturated hygrometry.

METHODS

- Samples analyses were performed just after injection (T0) or 24 hours later (T24)
- Non-injected samples were also analyzed at T0 and T24 as control samples.
- Analysis was performed by:
  1. Ultrasound (Dermo-echography), and
  2. Histopathology

METHODS

1. Ultrasound (Dermo-echography):
   - The ultrasound measurements were performed with a DermaScan C v.3 (a registered trademark of Cortex Technology, Monaderm, Monaco), equipped with a 12 mm probe (maximum depth 10 mm) working at 20 MHz with an axial resolution of 60 µm and lateral resolution of 130 µm.

   - After acquisition and registration, the ultrasound images were processed, and the skin thickness was measured for each ultrasound images. The mean of the three values obtained for each condition was used for the analysis.

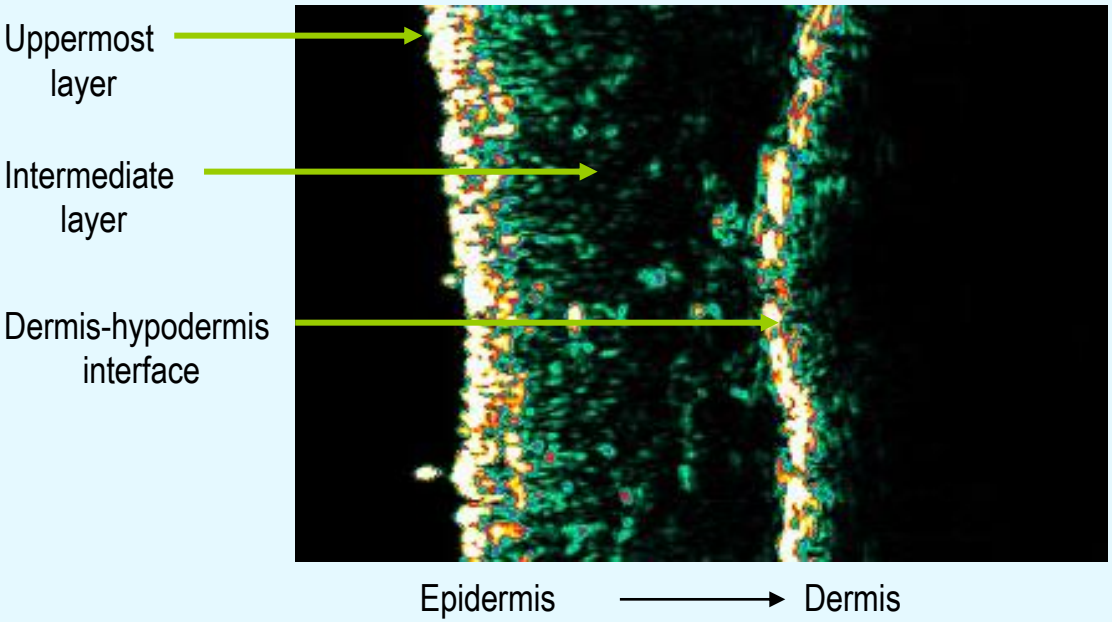
METHODS

2. Histopathology:
   - Fixation: 10% buffered formaldehyde
   - Trimming and embedding: each sample cut in 2, both halves in the same block
   - Dehydratation/ impregnation/ embedding: in paraffin wax, routine method
   - Microtomy: 4-µm thick section (one slide per block)
   - Staining: HE (hematoxylin-eosin) and PAS (periodic acid Shiff)-alcian blue staining (specific for hyaluronic acid).
   - Image capture: slides were scanned using Mirax™ scan system from Zeiss
   - Image analysis: After color deconvolution and use of a binary mask in Matlab®, surface areas (mm^2) were measured

RESULTS

20 MHz ultrasound images of *ex vivo* human skin samples

Ultrasound image of *ex vivo* skin sample before injection of dermal filler



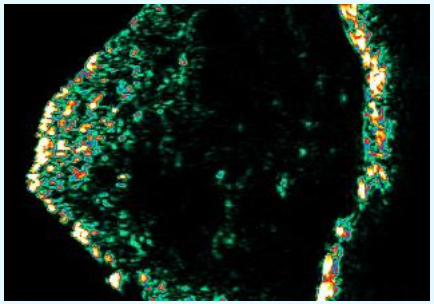
- Ultrasound image showed three layer structures:
  1. Uppermost layer: echogenic
  2. Intermediate layer: poorly echogenic
  3. Dermis-hypodermis interface: echogenic

RESULTS

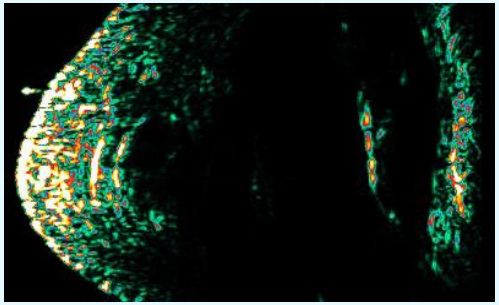
20 MHz ultrasound images of *ex vivo* human skin samples

Ultrasound images of *ex vivo* skin sample after injection of dermal filler

Injection volume: 100 µL

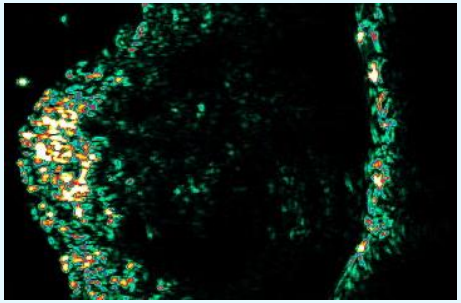


Injection volume: 200 µL

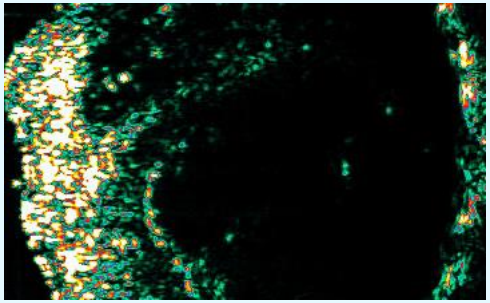


Ultrasound images just after dermal filler injection (T0)

Injection volume: 100 µL



Injection volume: 200 µL

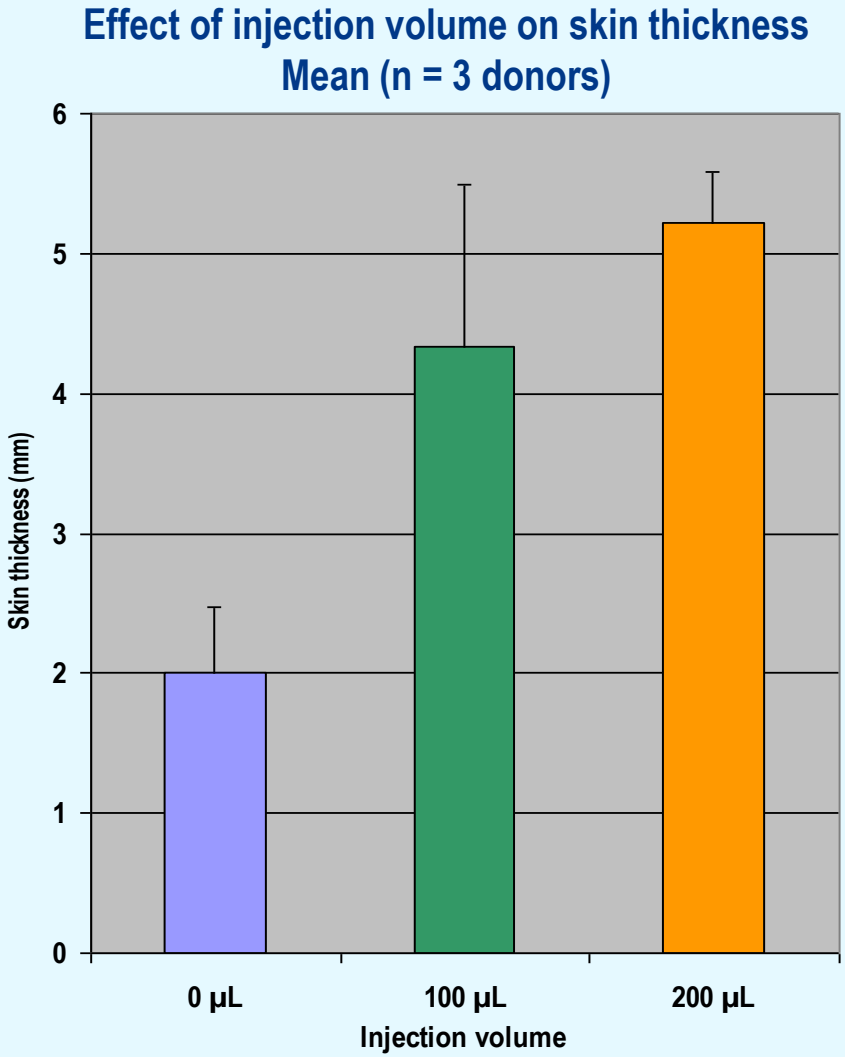


Ultrasound images 24 hours after dermal filler injection (T24)

- Ultrasound images after dermal filler injection showed that:
  1. The dermis was largely thickened
  2. The dermis appeared less echogenic
  3. In some cases, totally non-echogenic zones were observed within the dermis probably corresponding to hyaluronic acid that is non-echogenic.

RESULTS

Ultrasound image analysis of the effect of dermal filler injection on skin thickness in *ex vivo* human skin samples

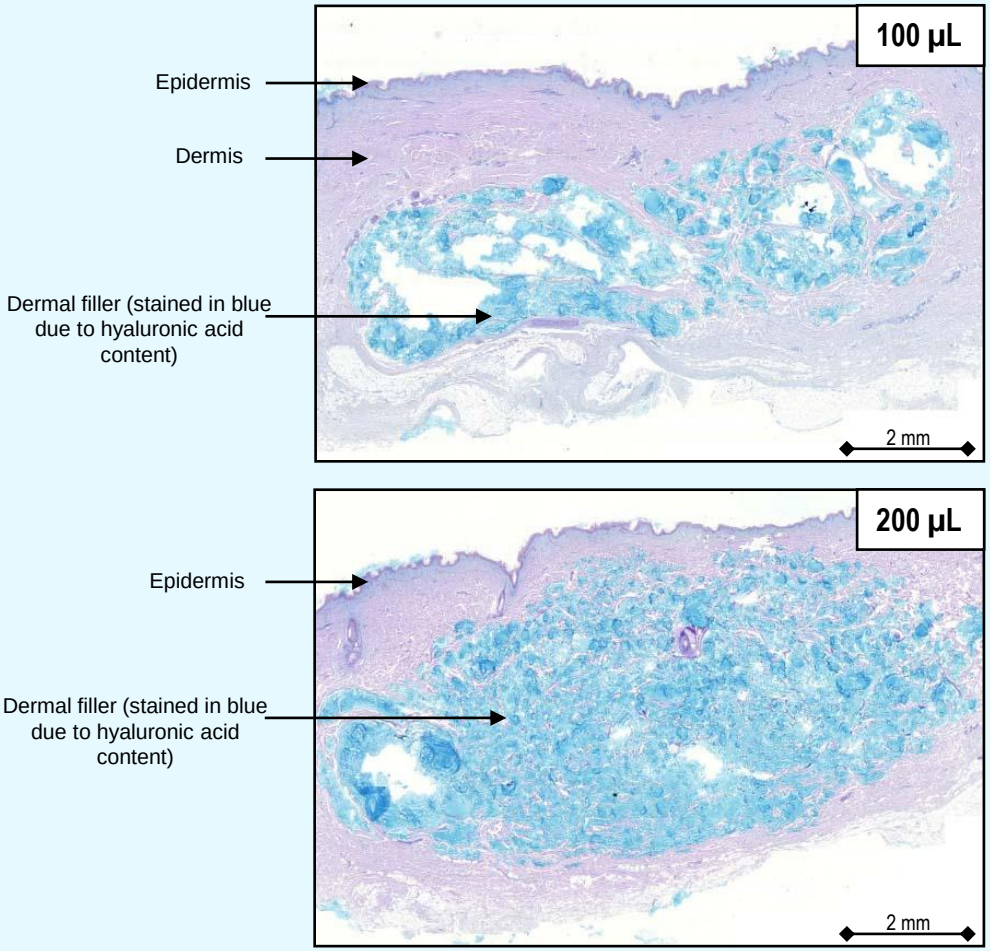


- Dermal filler injection in *ex vivo* human skin induced a marked skin thickness increase that can be estimated by image analysis (Dermascan Software).
- Relative to the concurrent control (0 µL), skin thicknesses increased by a 2.2-fold factor after 100 µL injection and by a 2.6-fold factor after 200 µL injection at T0 (n = 3 donors).
- For each donor, the skin thicknesses were very reproducible within each of the 3 injections (CV < 10%).
- Similarly, there was a low inter-individual variability in skin thickness, with CV ranging between 7% and 27%.

RESULTS

Histopathological analysis of dermal filler in *ex vivo* human skin samples

Microphotographs of *ex vivo* human skin
Injection volumes 100 and 200 µL, Donor 3 at T24h



- At microscopic examination, the sites of injection is well visible at low magnification in the dermis.
- Dermal filler is clearly visible as it is stained in blue due to hyaluronic acid content.
- Measurement of the area (mm^2) occupied by the dermal filler was performed by image analysis after color deconvolution and use of a binary mask in Matlab®. A statistically significant discrimination was possible between 100 and 200 µL injection volumes ($p < 0.001$).

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CONCLUSION

Dermo-echography and histopathology evaluation techniques associated with image analysis are suitable to monitor *in vitro* human skin thickness and morphology changes due to dermal filler injections. This cost-effective model allows simple, rapid and reproducible visualization of dermal fillers.