

# IN VITRO VISUALIZATION OF HYALURONIC ACID DERMAL FILLER INJECTION IN HUMAN SKIN: COMPARISON BETWEEN THREE DIFFERENT FILLERS

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## 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.
- Dermal filler properties differ both between and among classes. Hyaluronic acid (HA) based fillers have varied life spans ranging from weeks to months depending upon their degree of reticulation.
- Highly reticulated HA fillers are more resistant to *in situ* degradation and show clinical efficacy for up to 1 year.
- The aim of this work was to compare the diffusion pattern of three dermal fillers only differing by their degree of reticulation injected in *ex vivo* human skin using dermo-echography and histopathology evaluation techniques associated with image analysis.

## METHODS

### Samples and filler

- Fresh human skin samples obtained after abdominal surgical procedures from three different donors were used.
- Three intradermal injection of hyaluronic acid-based dermal filler Emervel<sup>®</sup> were performed on each skin sample.
- Dermal fillers tested:
  - Emervel<sup>®</sup> Deep (highly reticulated).
  - Emervel<sup>®</sup> Classic (moderately reticulated).
  - Emervel<sup>®</sup> Touch (slightly reticulated).
- Dermal filler injection volume: 100  $\mu$ L.
- After injection, skin samples were maintained during 24 hours in organo-culture medium and kept at 37°C, 95% CO<sub>2</sub> and saturated hygrometry.

### Analysis performed

- 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:
  - Ultrasound (Dermo-echography).
  - Histopathology with image analysis.

### 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  $\mu$ m and lateral resolution of 130  $\mu$ m.
- After acquisition and registration, the ultrasound images were processed, and the skin thickness was measured for each ultrasound images (Dermascan software). The mean of the three values obtained for each condition was used for the analysis.

### HISTOPATHOLOGY WITH IMAGE ANALYSIS

- Fixation: 10% buffered formaldehyde.
- Trimming and embedding: each sample cut in 2, both halves in the same block.
- Dehydration/ impregnation/ embedding: in paraffin wax, routine method.
- Microtomy: 4- $\mu$ m thick section (one slide per block)
- Staining: HE (hematoxylin-eosin) and PAS (periodic acid Schiff)-alcian blue staining (specific for hyaluronic acid).
- Image capture: slides were scanned using Mirax<sup>™</sup> scan system from Zeiss.

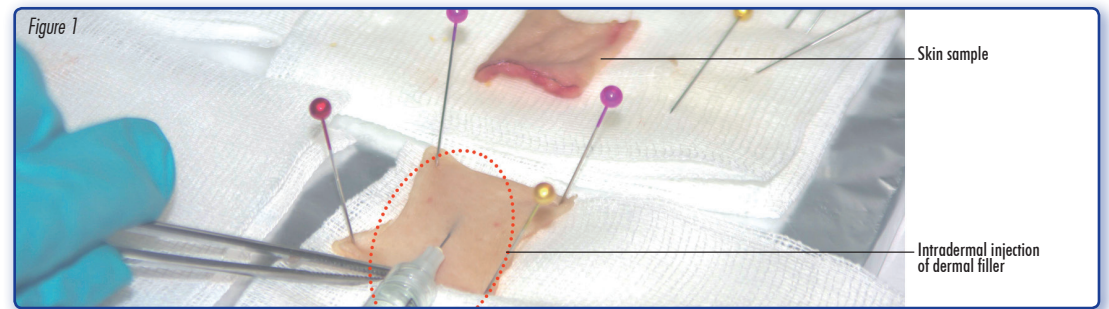
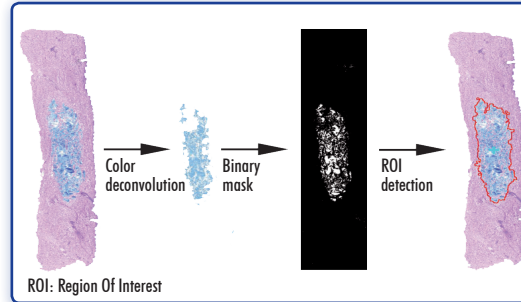


Figure 2 & 3 Image analysis

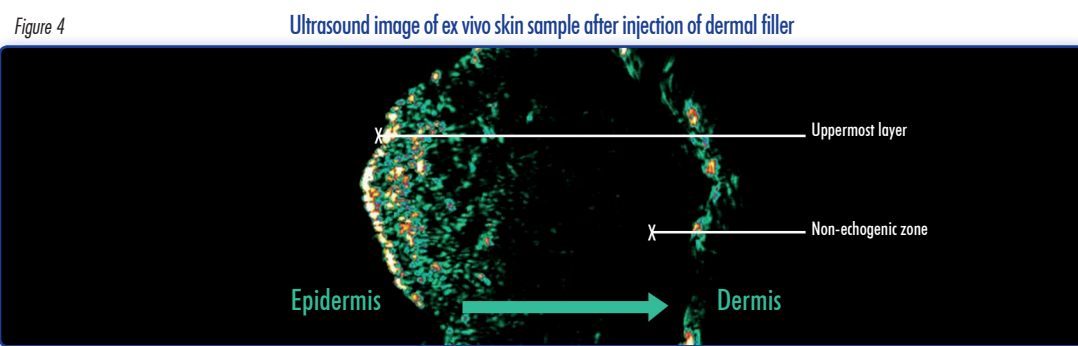


$$\text{Texture} = \frac{\text{Filler (blue component in ROI)}}{\text{Filler + Tissue (blue + pink components in ROI)}}$$

- After color deconvolution and use of a binary mask in Matlab<sup>®</sup>, the surface of the injected area and its texture were measured.
- The texture parameter is an objective measurement of the filler behavior in the dermis: it describes the degree of entanglement of the filler material in the dermal tissue.

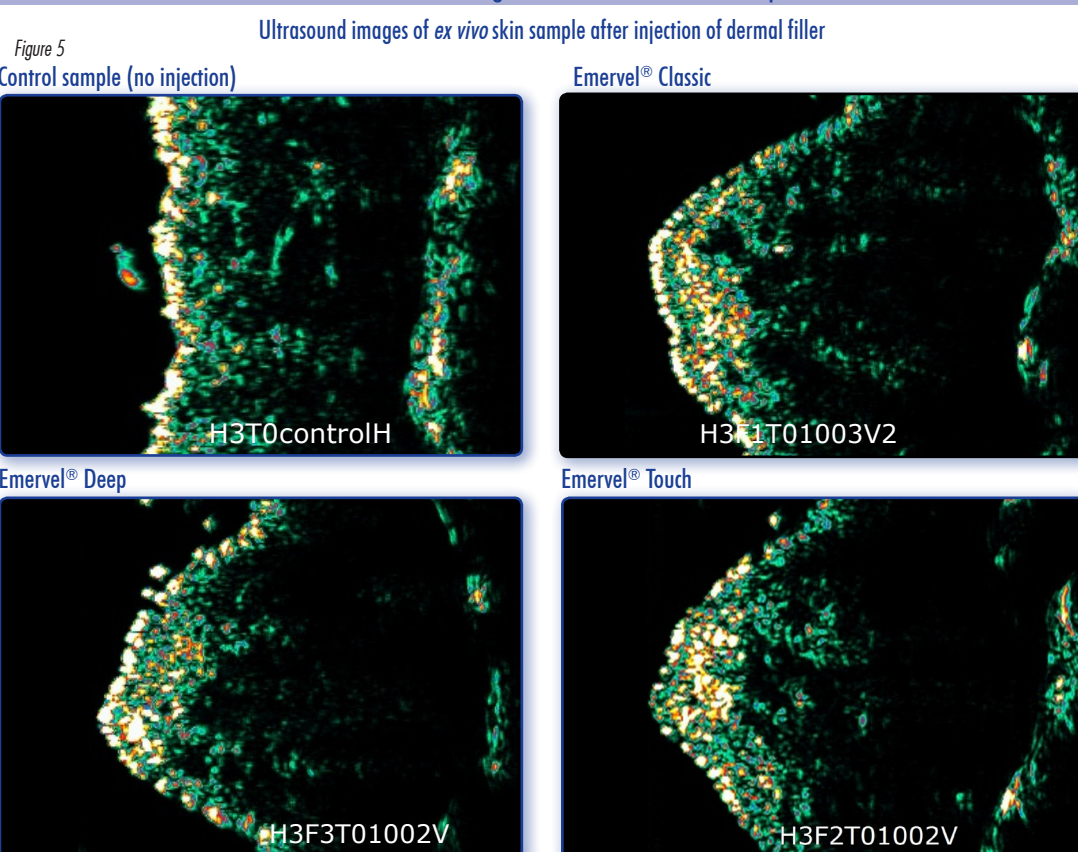
## RESULTS

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



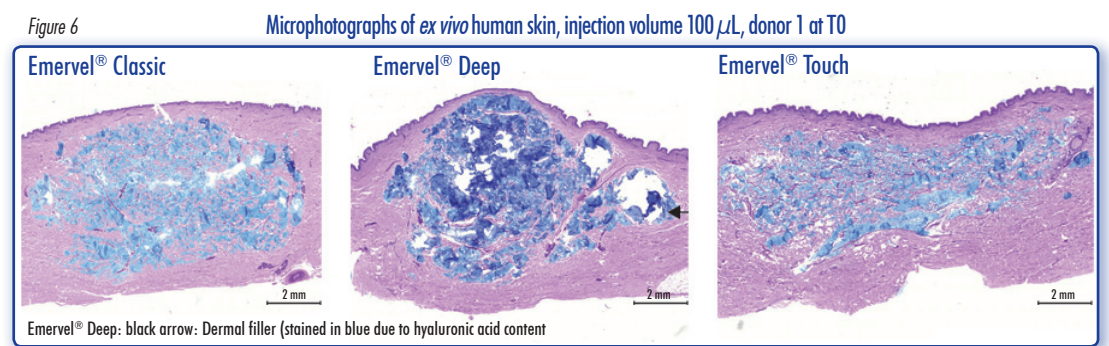
- Ultrasound image showed three layer structures:
  - Uppermost layer: echogenic
  - Intermediate layer: poorly echogenic
  - Dermis-hypodermis interface: echogenic.
- After injection of Emervel<sup>®</sup>, the dermis was largely thickened and appeared less echogenic.

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



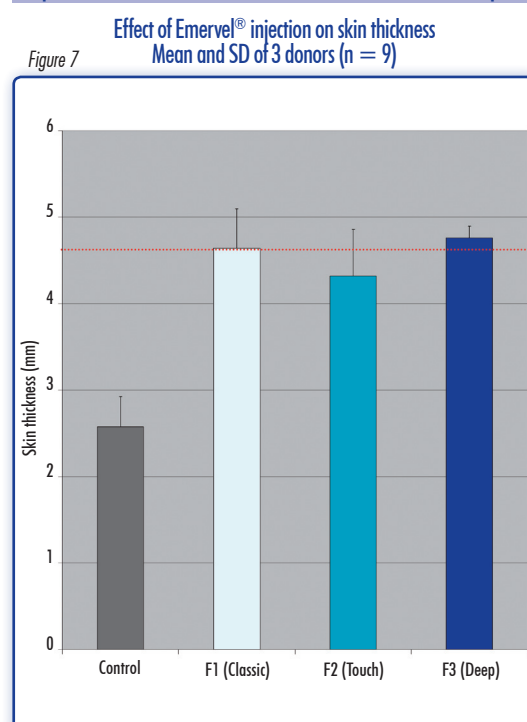
- Ultrasound images just after dermal filler injection (T0)
- Ultrasound images after injection of Emervel<sup>®</sup> dermal filler showed that:
  - The dermis was largely thickened.
  - The dermis appeared less echogenic.
- With the three dermal filler of Emervel<sup>®</sup> range, totally non-echogenic zones were observed within the dermis probably corresponding to the dermis area occupied by hyaluronic acid (non-echogenic material).

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



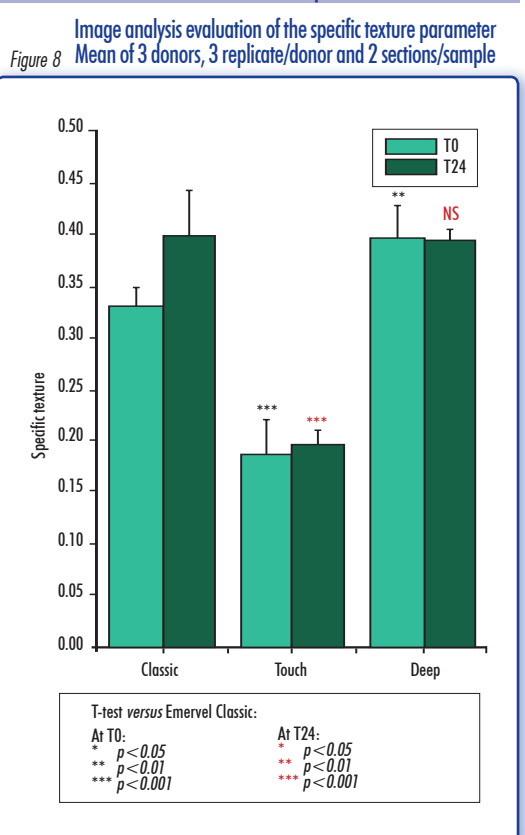
- At microscopic examination, the sites of injection are well visible at low magnification in the dermis: the three dermal fillers appeared in blue due to their hyaluronic acid content.

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



- Dermoechographic examination showed a very reproducible skin thickness increase (CV < 5%) with the three fillers tested.
- The lower increase was observed with Emervel<sup>®</sup> Touch (the less reticulated filler), though not reaching statistical significance.
- This tendency was more visible 24 hours after dermal filler injection, probably due to a rapid diffusion of the less reticulated filler.
- In the case of Emervel<sup>®</sup> Classic, skin thickness increased by 1.80-fold compared to control non injected skin samples.

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



- Using the texture parameter, the image analysis showed a statistically significant differences between the control and each of the three dermal fillers tested ( $p < 0.001$ ).
- In the case of Emervel<sup>®</sup> Touch (lower reticulation), the filler material appears more entangled within the surrounding tissue than with the two other fillers.

## CONCLUSIONS

*In vitro* excised human skin model, combining dermo-echography and histopathology evaluation techniques associated with image analysis, is suitable to compare dermal fillers only differing by their degree of reticulation on a short-time period.