HISTOLOGICAL EVALUATION OVER TIME OF FOUR HYALURONIC ACID BASED DERMAL FILLERS

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BACKGROUND

TEOXANE Laboratories developed a new line of hyaluronic acid dermal fillers, TEOSYAL®RHA (Resilient Hyaluronic Acid®), based on an exclusive "preserved network" technology using less BDDE* and with higher stretch and strength properties, specifically dedicated to suit the dynamic areas of the face. In this study, the immediate and short term tissue integration of TEOSYAL®RHA is compared to three other commercially available reference products.

OBJECTIVE

The objective of this study was to assess by histological analysis the early evolution of the tissue integration of TEOSYAL® RHA 4 compared to three commercially available dermal fillers with similar indications for creation/restoration of facial volume by deep dermal or subcutaneous injections.

MATERIALS AND METHODS

Eight New Zealand White rabbits were injected subcutaneously with the four investigated products (6 injections of 300 µL per animal, *Figure 1*) for a total of 12 injections per product[1]:

- **A TEOSYAL® RHA 4 (TEOXANE, product A),**
- **B TEOSYAL® PureSense Ultra Deep (TEOXANE, product B),**
- **C Juvéderm® Voluma with Lidocaine (ALLERGAN, product C),**
- **D Restylane Perlane™ Lidocaine (GALDERMA, product D).**

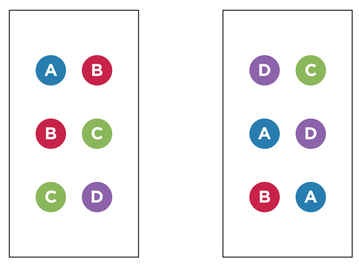

Figure 1. Representative pattern of injection at one of the time points.

At 3 days, 7 days, 14 days and 30 days post injection, the products' nodules of two animals were sampled with the surrounding tissues including all the skin layers up to the subcutaneous muscle (3 nodules / product / time point). Histological slides were stained using the following agents: Sirius red (*Figure 3*), Hematoxylin Eosin Safranin (HES, *Figure 5a*) and Masson's trichrome (*Figure 5b*). Evaluations included the collagen density and fibrosis around the injection site as well as the qualitative and semi-quantitative histologic evaluation of the local tissue effects and inflammatory response.

RESULTS

Nodules sampling

46 of 48 nodules and implantation sites were positively identified and considered adequate for histological evaluation. Almost all nodules had an ovoid shape when sampled. Product A presented a few more cases of flattened ovoid shape when sampled.

Evolution of collagen with time

Collagen density was assessed from Sirius Red colored histological slides. Evolutions with time of the collagen density around the product are presented in *Figure 2*. Collagen production around the implants matched with a classical body response showing a regular increase before reaching a plateau (here from D14). The results at D03 are uncertain since some dermal filler material was lost during histological slides preparation with product B, C and D. At 30 days after injection, all products yielded to a collagen layer which seemed to be thicker and more organized for the products C and D, as illustrated by the representative photographs of *Figure 3*. The global fibrosis development was less prominent at injection sites of product A than for the other injection sites.

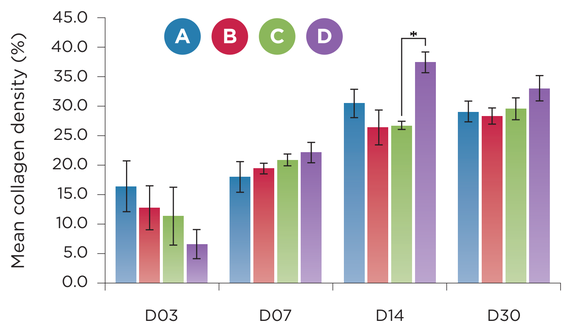

*Figure 2. Evolution with time of collagen around the product (mean values and SEM**).*

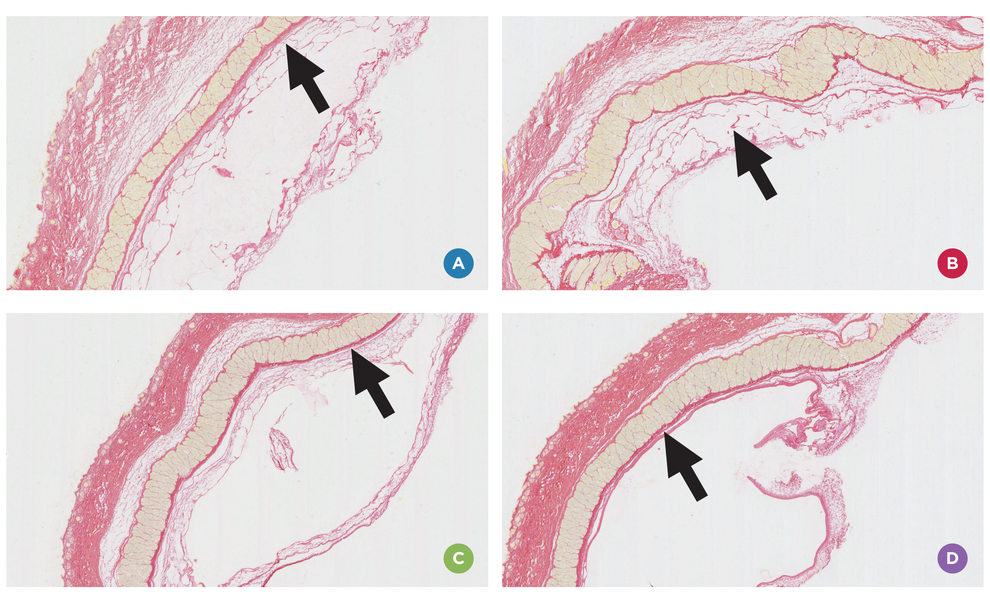

Figure 3. Representative photographs of Sirius red coloration of the collagen proteins in the histological slides, 30 days after implantation of each products (mag. x1.25). The black arrows highlight the collagen fibers network around the products, which appears to be looser around products A and B, and thicker and more organized around the products C and D.

Inflammatory cell types evolution with time

From the HES (*Figure 5a*) and Masson's trichrome (*Figure 5b*) colored histological slides, semi-quantitative scores were used to describe microscopic findings and particularly the level of inflammatory cells: 0 (absent), 1 (slight), 2 (moderate), 3 (marked) and 4 (severe). *Figures 4a, b and c* show the evolution of three main inflammatory cell types with time for each product.

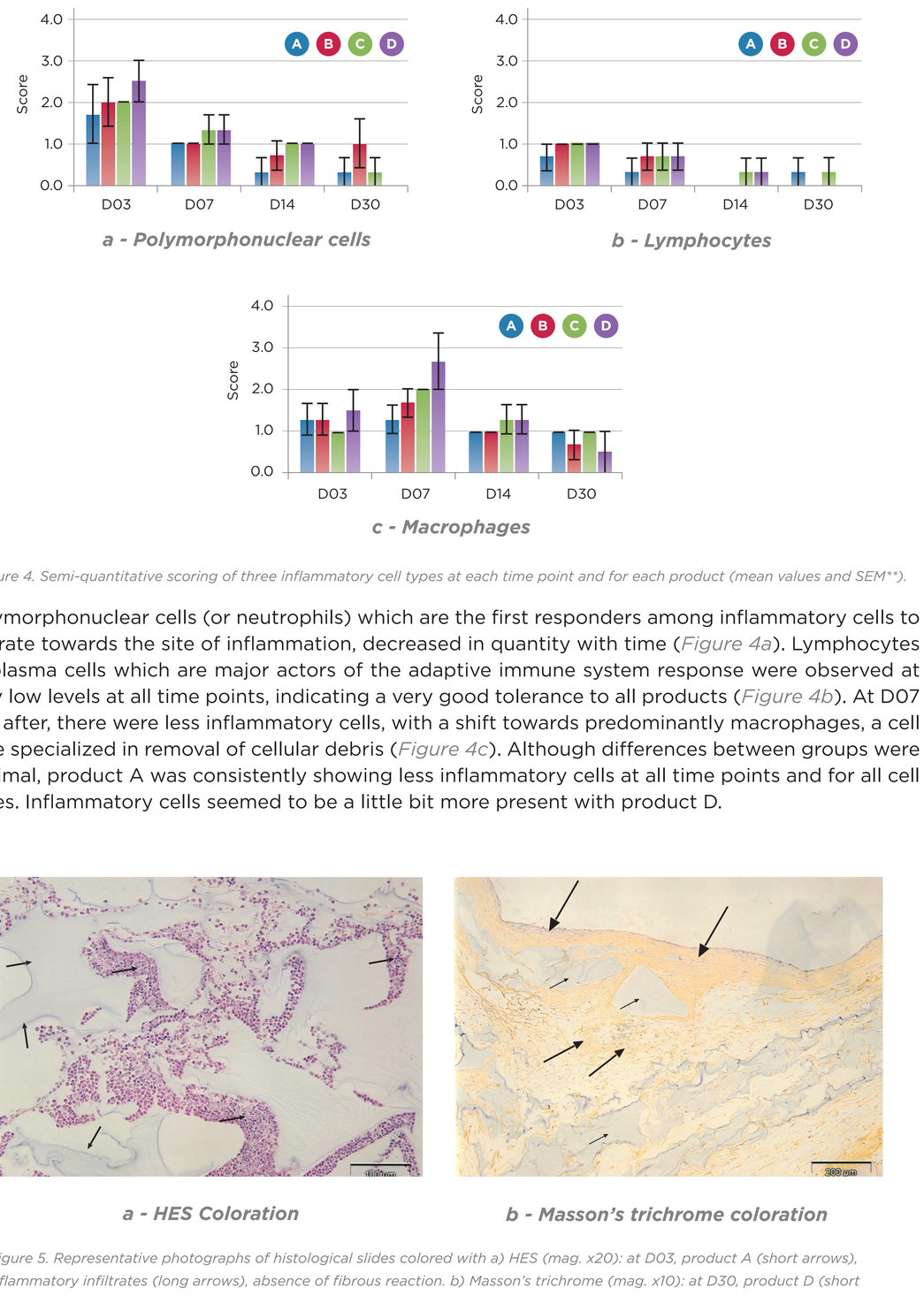

*Figure 4. Semi-quantitative scoring of three inflammatory cell types at each time point and for each product (mean values and SEM**).*

Polymorphonuclear cells (or neutrophils) which are the first responders among inflammatory cells to migrate towards the site of inflammation, decreased in quantity with time (*Figure 4a*). Lymphocytes or plasma cells which are major actors of the adaptive immune system response were observed at very low levels at all time points, indicating a very good tolerance to all products (*Figure 4b*). At D07 and after, there were less inflammatory cells, with a shift towards predominantly macrophages, a cell type specialized in removal of cellular debris (*Figure 4c*). Although differences between groups were minimal, product A was consistently showing less inflammatory cells at all time points and for all cell types. Inflammatory cells seemed to be a little bit more present with product D.

Figure 5. Representative photographs of histological slides colored with a) HES (mag. x20): at D03, product A (short arrows), inflammatory infiltrates (long arrows), absence of fibrous reaction. b) Masson's trichrome (mag. x10): at D30, product D (short arrows), slight fibrosis (long arrows) and moderate fibrous encapsulation (long arrows).

Initial and chronic inflammation, nuclear debris

Inflammation reactions were minimal (very low levels of edema and hemorrhage) for all products and at all time points. On D03, D07 and D30 edema were present and it were more prominent for the product D.
No significant neovascularization was observed for any product from D03 to D30.
No cellular debris from inflammatory cells was seen for product A at any time points. Whereas minimal nuclear debris were observed at D03 and D07 for products B, C and D.

CONCLUSION

To the best of the authors' knowledge this is the first study aiming to precisely study the kinetic of the tissue response of a host within one month after a dermal filler injection. Four products indicated for creation/restoration of facial volume by deep dermal or subcutaneous injections were studied: TEOSYAL® RHA 4 (product A), TEOSYAL® PureSense Ultra Deep (product B), Juvéderm® Voluma with Lidocaine (product C), Restylane Perlane™ Lidocaine (product D). Throughout the course of the study, injections appeared to be well tolerated at all injection sites and with all materials. Although at a given time point differences between groups were minimal, a general trend in favor of TEOSYAL® RHA 4 (product A) was observed based on the results obtained on the 46 nodules analyzed. At each time point from D03 to D30, TEOSYAL® RHA 4 (product A) yielded to less prominent tissue reactions at injection sites than the 3 other dermal fillers tested and hence results suggest a better tissue integration. The results of this pilot study lead us to set up further investigations on a larger sample size, at a specific time point, to give enough power to achieve a statistically significant conclusion.

*BDDE : 1,4-Butanediol Diglycidyl Ether
**SEM : Standard Error of the Mean

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REFERENCES

1. Study Report N° P155A "Histological evaluation over time of hyaluronic acid based dermal fillers compared to reference products injected subcutaneously on rabbits." VOXCAN, January 20th, 2016.