Effect of a hyaluronic acid based filler on proliferation and differentiation of human preadipocytes and anti- lipolytic effect on human mature adipocytes

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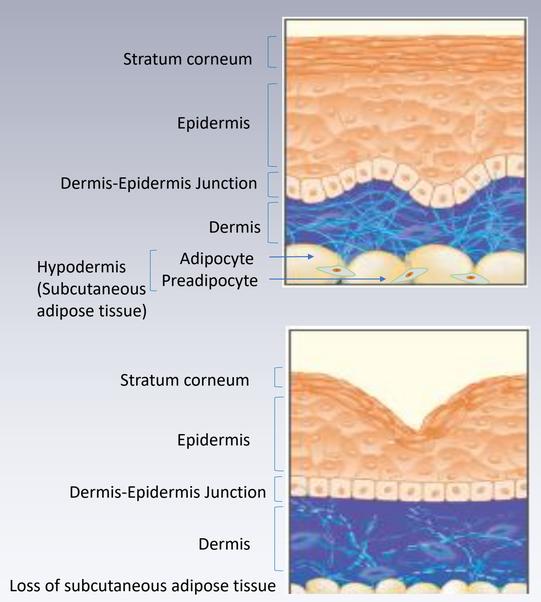
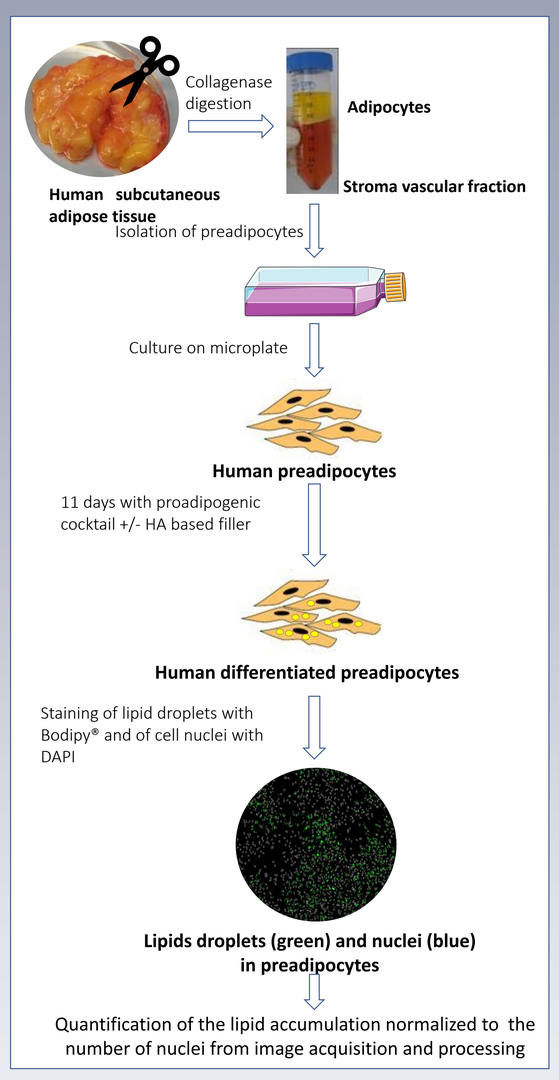
INTRODUCTION

Ageing is characterized by skin alterations and can be macroscopically recognized because of wrinkles, loss of elasticity and flexibility, sagging and thinning of the skin.



Notably a loss of subcutaneous adipose tissue is observed during ageing, resulting in deleterious effects on the appearance of the skin, specifically in the area of the face and neck where the effect of gravity is more obvious. With ageing, the volume of facial fat decreases because of a defective differentiation of adipocyte progenitors called “preadipocytes” and/or because of an accentuated lipolytic activity of mature adipocytes. Hyaluronic acid (HA) based fillers represent a well-known positive effect on the dermis component while filling the skin depressions. However there is no adequate information of the HA based filler effect on metabolic and morphologic aspects of the human adipose cells. This study is designed to verify these effects.

MATERIELS AND METHODS

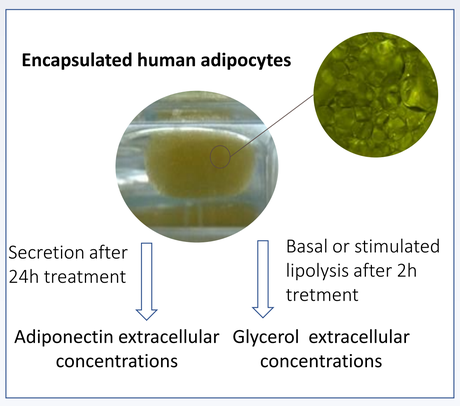


1. Preparation of human adipose cells

Subcutaneous adipose tissues biopsies were obtained from female patients undergoing aesthetic or reconstructive surgery. Adipose tissue was digested with collagenase and the digested material was filtered and centrifuged. The cells in suspension corresponding to adipocytes were washed before being encapsulated in a peptidic gel to maintain their survival and metabolism. The resulting pellet [stroma vascular fraction (SVF)] was filtered and was resuspended in DMEM-10% fetal bovine serum (FBS) after treatment with erythrocyte lysis buffer and washing in PBS. Preadipocytes (PA) were obtained for cell culture after one passage to eliminate non-preadipocyte cell contamination

2. Differentiation of preadipocytes

Preadipocytes were incubated with a proadipogenic cocktail including insulin, glucocorticoid and thiazolinedione and in presence or not of the HA based filler (ART FILLER® Volume, FILLMED, France) at two concentrations, 0.3% and 0.02%. The medium was changed every 2 days until 11 days. At the end of the culture period, cells were fixed and secretion media were collected. Lipid accumulation was quantified in cells after lipid droplets staining and the number of cells was determined after nuclei staining.



3. Adipocyte metabolism

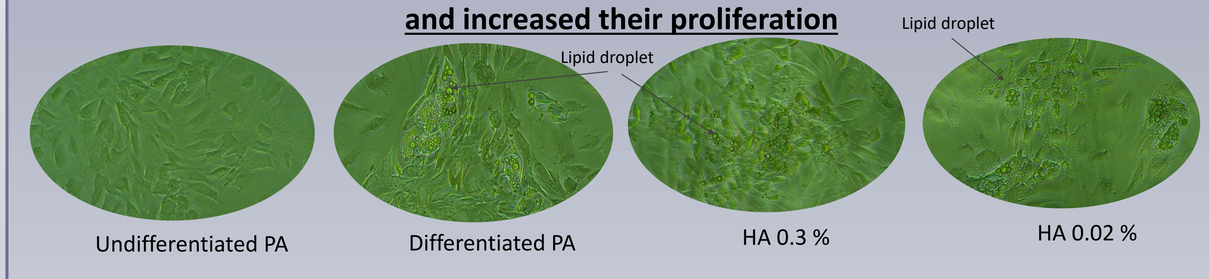
Encapsulated adipocytes were treated with or without the reference lipolytic effector, isoproterenol at 1uM and the HA based filler (ART FILLER® Volume, FILLMED, France) at two concentrations, 0.3% and 0.02% for two hours. After the treatment period, secretion media of cells were collected for quantification of glycerol extracellular concentrations with colorimetric dosage and adiponectin extracellular concentrations were quantified by ELISA assay.

RESULTS

The effect of the HA based filler at two concentrations 0.3% (HA 0.3 %) and 0.02% (HA 0.02%) was compared to the control condition corresponding to cells treated only with an adipogenic cocktail (Differentiated PA).

When cultivated with both proadipogenic cocktail and the HA based filler, we observed that the preadipocytes maintained their capacities to accumulate lipid droplets, one marker of preadipocyte differentiation (**figure 1**) in comparison to differentiated PA. However, the preadipocytes treated with the HA based filler (ART FILLER® Volume, FILLMED, France) at 0.3% accumulated lipid droplets only from one week of culture suggesting a later commitment in adipogenesis. This result is associated with a proliferative effect of the HA based filler on the preadipocytes during the first week of culture. The data showed a dose dependent effect and a significantly increase of nuclei number up to 93% (**figure 2**).

The HA based filler preserved the lipid accumulation capacities of preadipocytes and increased their proliferation



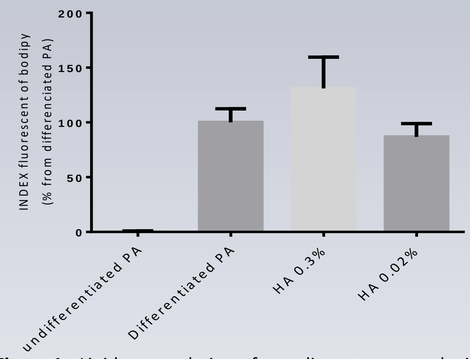


Figure 1: Lipid accumulation of preadipocytes treated with or without the HA based filler at 0.3% (HA 0.3%) and 0.02% (HA 0.02%).

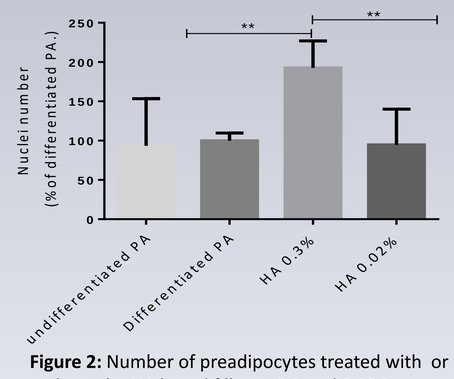


Figure 2: Number of preadipocytes treated with or without the HA based filler at 0.3 and 0.02 %

The HA based filler decreased the lipolytic activity of adipocytes

The HA based filler inhibited at a dose dependent manner the basal lipolysis of mature adipocytes (**figure 3**) as well as their lipolytic activity stimulated by isoproterenol (**figure 4**). Whatever the concentration of HA based filler, we observed (**figure 5**) that the adiponectin level was maintained, reflecting the preservation of adipocyte metabolism.

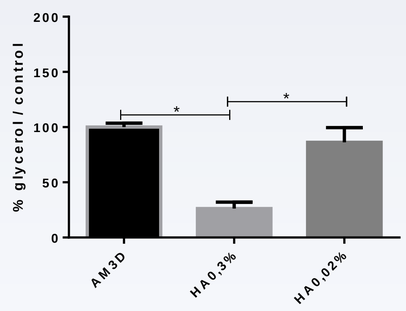


Figure 3: Basal lipolysis of adipocytes treated with or without the HA based filler

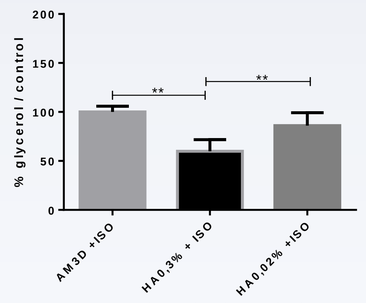


Figure 4: Stimulated lipolysis of adipocytes treated with or without the HA based filler

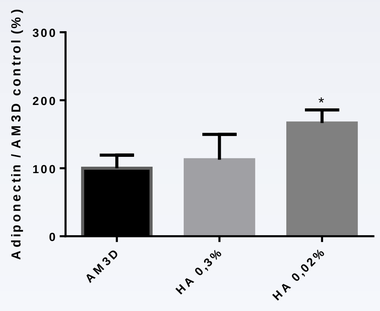


Figure 5: Adiponectin secretion of adipocytes treated with or without the HA based filler

CONCLUSION

In our study, we demonstrated for the first time in human adipose cells that a hyaluronic acid based filler (ART FILLER® Volume, FILLMED, France) induced the proliferative capacity of adipocyte progenitors while preserving their differentiation. Moreover, it was able to decrease the basal and stimulated lipolysis of mature adipocytes while maintaining their metabolism, notably the secretion of adiponectin. **The hyaluronic acid based filler represents a potential candidate for preventing the deleterious effects of ageing in skin and in the loss of facial hypodermis**.

To complete these first results, we will evaluate the capacity of the hyaluronic acid based filler to create a microenvironment conducive to a better differentiation of preadipocytes and to a prolonged survival of these cells. Thus, human preadipocytes will be treated with the HA based filler beyond 11 days, up to 20 days of culture, and effects of the product will be evaluated on proliferation rate and lipid accumulation in these cells.

References:
M. Keophiphath et al., 1,2-Vinyldithiin from Garlic Inhibits Differentiation and Inflammation of Human Preadipocytes, *The Journal of Nutrition*, Volume 139, Issue 11, 1 November 2009, Pages 2055–2060
V. Pellegrinelli et al., Endothelial Cells From Visceral Adipose Tissue Disrupt Adipocyte Functions in a Three-Dimensional Setting: Partial Rescue by Angiopoietin-1; *Diabetes* Feb 2014, 63 (2) 535-549