3D BIOPRINTED HUMAN ENDOTHELIALIZED ADIPOSE TISSUE AS A NEW PREDICTIVE MODEL FOR *IN VITRO* EVALUATION

Poster 569

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Introduction

Current tissue-engineered skin models lack of the **hypodermis** which has specific **metabolic and functional properties**[1,2]. Adipose tissue stem cells (ASCs) are essential because adipocytes do not proliferate[3].

The **3D bioprinting approach** combined with advanced tissue engineering, provides a potential solution for the reconstruction of a functional 3D hypodermis.

We developed a 3D bioprinting approach with the goal of creating physiological and functional biological adipose tissue. To get as close as possible to the physiological conditions, we engineered an adipose tissue construct with **microvascularisation** by adding endothelial cells.

We finally assessed the **functionality** through two essential functions: **lipogenesis** and **lipolysis**. For lipolysis induction, we chose two highly described adrenergic stimulants: caffeine and isoproterenol.

Materials & Methods

ASC combined with human **hypodermal microvascular endothelial cells** (HHMVEC) were mixed in a **patented bioink** and printed with optimal printing conditions and printer functions[4]. For the development of the microvascularisation, the bioink was functionalized with a cocktail of endothelial growth factors. We compared the effect of this supplement directly into the culture medium or added into the bioink (**functionalized bioink**) with a not supplemented control.

To assess adipocytes functionality, we selected and tested adipogenic modulators promoting lipogenesis (oleic acid) and lipolysis (caffeine and isoproterenol).

References

1. *Bullough W. S, et al. (1959), Exp. cell research.*
2. *Gregoire F, et al. (1998), Physiological review.*
3. *Lequeux C, et al. (2012), Skin Pharmaco. and Physiol.*

Acknowledgements

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Results & Discussion

1. 3D Bioprinted endothelialized adipose tissue is morphogically consistent with numerous mature adipocytes containing large vacuoles expressing FABP4 and filled with lipids

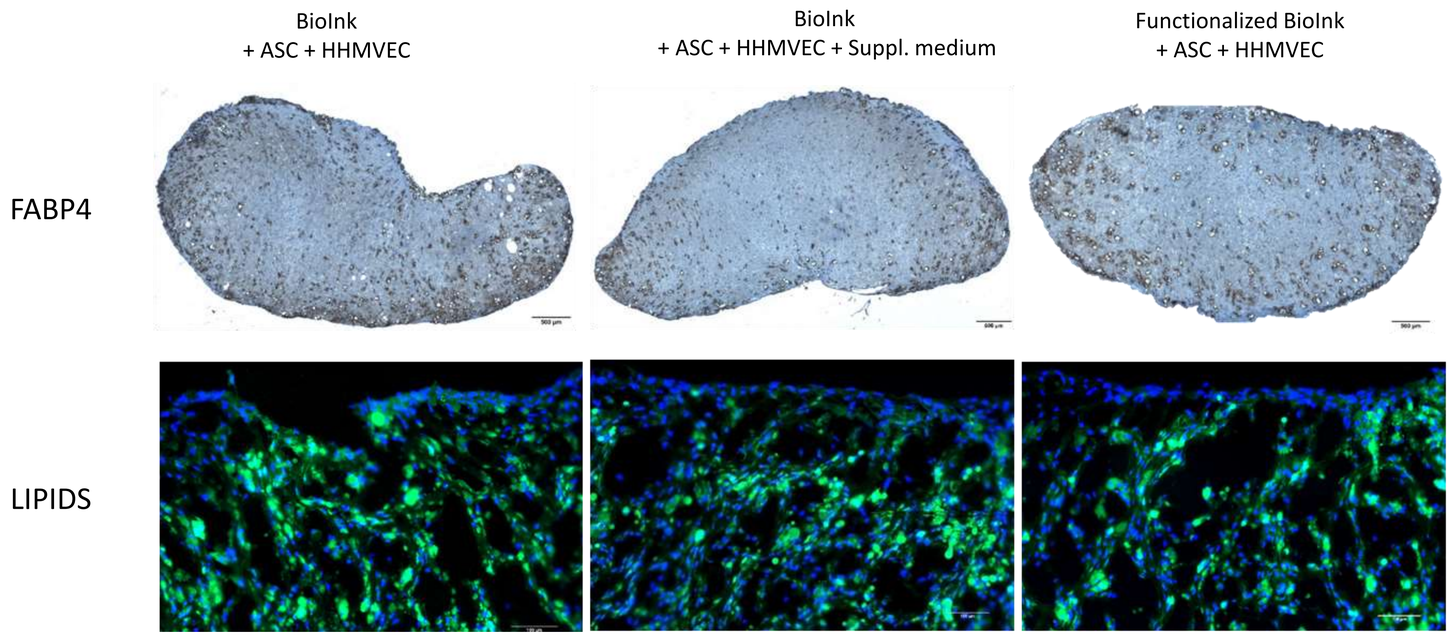

Figure 1: FABP4 (Fatty acid binding protein-4) immunostaining and bodipy staining of 3D vascularized adipose tissue reconstructed in a bioink +/- supplemented with endothelial growth factors (functionalized) or cultured +/- with endothelial growth factors supplemented in the cell culture medium.

2. 3D Bioprinted endothelialized adipose tissue shows capillary-like microvascular network surrounded by adipocytes

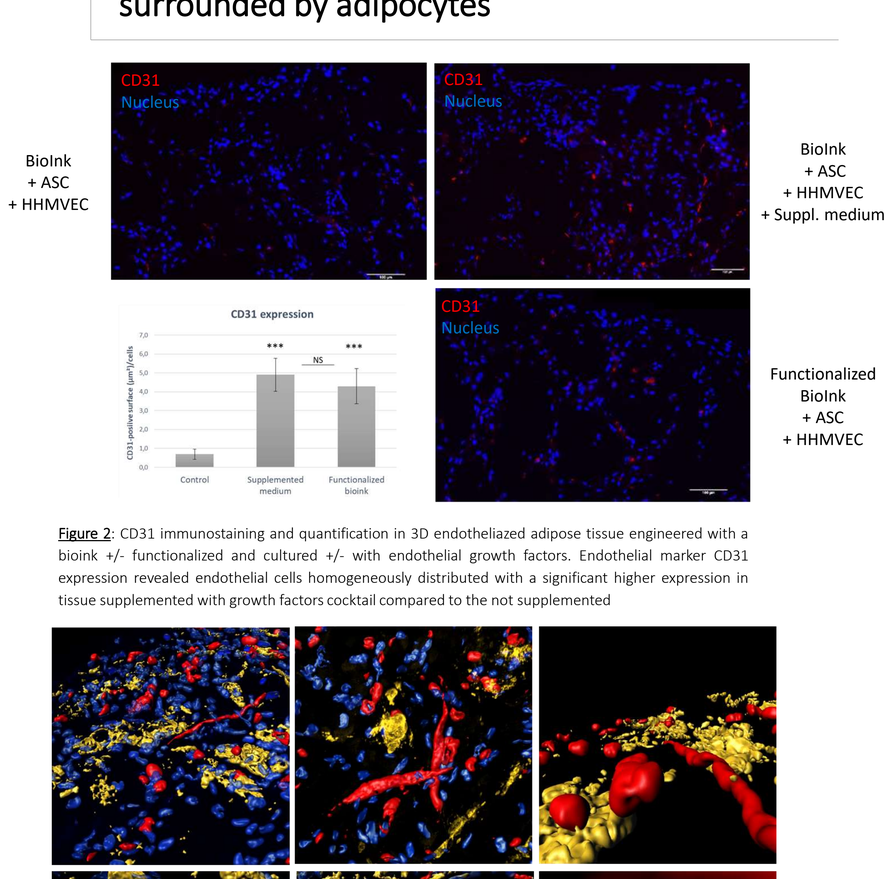

Figure 2: CD31 immunostaining and quantification in 3D endotheliazed adipose tissue engineered with a bioink +/- functionalized and cultured +/- with endothelial growth factors. Endothelial marker CD31 expression revealed endothelial cells homogeneously distributed with a significant higher expression in tissue supplemented with growth factors cocktail compared to the not supplemented

Figure 3: 3D confocal microscopy analysis of 3D bioprinted endotheliazed adipose tissue showing perilipin (yellow) and CD31 (red) expression (nucleus in blue). Co-staining of perilipin and CD31 showed the formation of an capillary-like microvascular network surrounded by adipocytes

3. 3D Bioprinted endothelialized adipose tissue demonstrates a functional lipogenesis/lipolytic activity

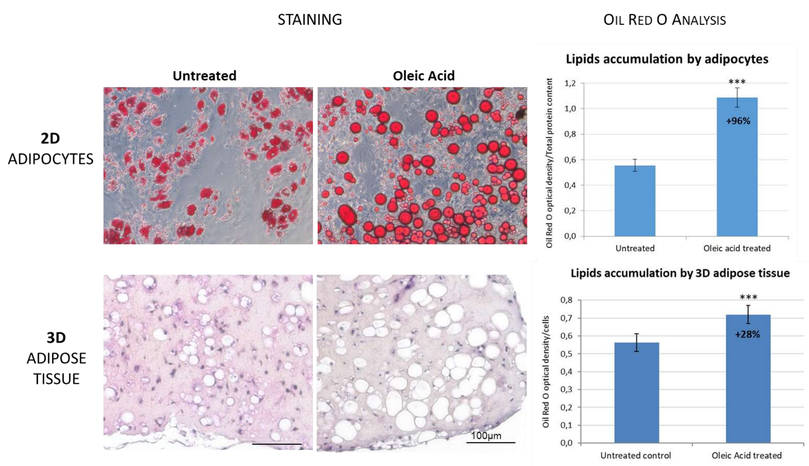

Figure 4: Study of oleic acid supplementation effect on adipocytes and reconstructed adipose tissue by microscopic and Oil-Red-O analysis. The addition of oleic acid demonstrated a proper **lipogenesis function** in 3D with a significant increase of the lipid vacuoles size observed in HPS and a 28% significant increase of lipid accumulation.

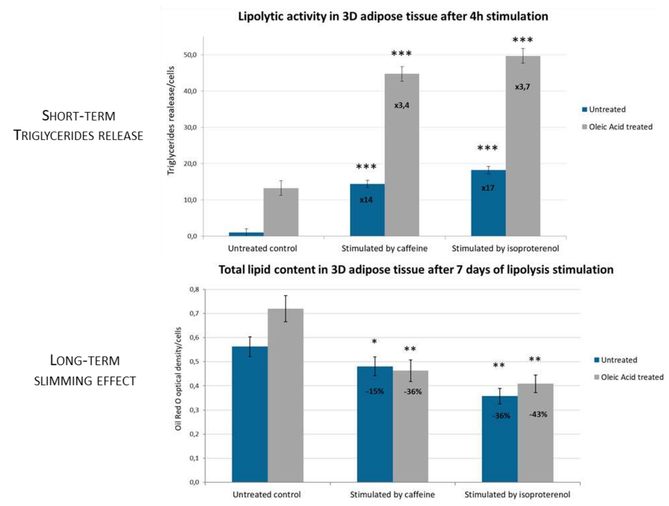

Figure 5: Study of the short and long-term lipolytic effect of caffeine and isoproterenol on the reconstructed adipose tissue. After adrenergic stimuli with caffeine and isoproterenol, adipose tissue demonstrated their ability to release lipids after a short-term or a long-term stimulus demonstrating a **lipolytic activity.**

Conclusions

For the first time, we have developed a new **bioprinting method for the generation of endothelialized human adipose tissue** with cellular and molecular characteristics, and functions closely resembling native human tissue. This unique *in vitro* model based on a **new functionlized bioink** may be a relevant tool to explore molecular mechanisms underlying **adipogenesis** and to **identify dermo-cosmetic active compounds**.