INTRODUCTION AND AIM OF THE STUDY

The hair follicle, a skin appendage, is a self-renewing "mini-organ" presenting several biological functions, such as skin repairing during re-epithelisation, dispersion of sweat and sweat gland products (e.g. pheromones and sebum). Moreover, it has a psychological and social importance in our society, so the study of its physiology and the development of hair-care products shows an increasing interest.

Hair follicle undergoes to a continuous cycles of growth (anagen phase) and regression (catagen phase) which imply deep morphological and physiological modifications. The reconstruction process starts in the bulb from dermal papilla (DP), a spherical core of mesenchymal cells, and subsequently involves the surrounding epithelial cells of the follicle. The molecular mechanisms underlying the HF cycle are complex and not fully understood but it is clear that the epithelium-mesenchymal interactions, and in particular the cross-talk between dermal papilla (DP) fibroblast and the keratinocytes of the outer root sheath (ORSK) play a pivotal role.

A DPF-ORSK co-culture model allows to investigate the mechanisms that regulate the HF cycle and to study the effects of active compounds in pre-clinical research. As reported in the study of Higgins et al. (2013, PNAS), dermal papilla cells deeply modify their gene expression profile when cultured as monolayer, but the transcriptional pattern can be partially restored when they are cultured as 3D spheroids. This draws the attention to the fact that the spatial distribution of the cells is fundamental in order to produce a verisimilar model and the use of spheroidal co-culture of DP and ORSK is the optimal method, because it doesn't include scaffold, to create a "proto-follicle". The aim of the present study was to create a spheroidal 3D model of hair follicle bulb (3D HF-MTS) based on the "hanging drop" method and to investigate its relevance and reliability for research studies and applications in cosmetics and dermatology. A multi-parametric approach was adopted in order to characterise the model at morphological and molecular level and to have a comprehensive view of its evolution during culture time.

The culture of derma papilla fibroblast in 3D spheroids partially restores the transcriptional profile of intact demapo-papilla (22% of totally differentially regulated transcripts) after passages in monolayer.

METHODS AND EXPERIMENTAL DESIGN

Human Hair Follicle Dermal Papilla Cells (HDPC) and Human Hair Follicle Keratinocytes (HHFK) supplier Immotrop-Spain have been used to reconstruct proto-hair microspheres using the hanging drop method into GRAVITYiplus plates from InSpheres (Fig.2a). The 3D HF-MTS have been further transferred in GRAVITYtemp for long term cultivation in a 7-days time course experiment. According to a dynamic and multi-parametric approach the 3D HF-MTS have been harvested at different time points (Fig.2b) and characterized on the basis of the following parameters:

- Viability by ATP measure (Cell titer Glow, Promega)
- Histo-morphological analysis: performed by Hematoxilin-Eosin (Histoline) staining of 7um formalin fixed paraffin embedded (FFPE) sections
- Immunostaining: performed on FFPE sections using anti-human CKS (Sigma) primary antibody and DAB chromogenic detection method (Life-technologies) or anti-human COLV (Santa Cruz) and fluorescent secondary antibody to monitor the presence and the maintenance of type-specific features. All the images have been captured using Leica Instruments (DM2500 microscope or SP2 confocal microscope) and LASX software.
- Gene expression: total RNA has been extracted from 3D HF-MTs per sample (RNAqueous kit, Life-technologies) and retro transcribed (RNAqueous kit, Life-technologies). The produced cDNA has been used for relative RTqPCR analysis by ΔΔCt method using primers BMP2, FG7, LAMC3 and GAPDH (as reference gene) TaqMan Assay and TaqMan Master mix in ABI7500 system Life-technologies. RTqPCR allowed to verify the restoration of transcriptional profile of specific genes, in comparison with 2D culture.

RESULTS

VIABILITY

CONCLUSIONS 1: VIABILITY AND MTS MORPHOLOGY

Viability of microtissue increases after at least 24h in gravity TRAP (T2) and stabilize until Ti (120H) indicating a good maintenance of metabolic functions. At day 7 the viability values are less reproducible and with higher variability indicating a dynamic behaviour of the proto-hair and a possible starting of a regressive phase.

The morphological analysis has revealed, as expected, a pluri-stratified structure of 3D HF-MTS in which a core of dermal papilla fibroblast is surrounded by a multi-layered stratum of follicular keratinocytes. The thickness of the epithelial stratum increases until day 3 and then stabilizes until day 6. At day 7 a pluristratification is slightly visible and a decrease in thickness seems to occur. As expected, the expression of CK6 is strong and specific in the epithelial layers composed by follicular keratinocytes (Havlickova 2008). The intensity of the staining and the thickness of the epithelial layers are marked and stable until day 3 then decrease until day 6. At day 7 no epithelial layer are stained. Collagen IV is the major component of basal which anchor epithelial strata on connective tissue and is present in hair follicle in the basement membrane zone between derma papilla and follicular keratinocytes (Liu et al. 2014). 3D proto-hair is localized to 3D folliculosis and keratinocytes and its expression decrease from day 3 to day 6.

CONCLUSION 2: 3 SPHEROID AS A MODEL OF A CYCLING HAIR BULB

In conclusion, we have developed a proto-hair model -3D HF-MTS that presents a well-defined tridimensional structure with a precise spatial distribution of the 2 cell types and a functional epithelium-mesenchyma interactions. It can be cultured up to 7 days, mimicking the hair bulb and presenting morphological features similar to an active (anagen-like) dermal papilla and a potential regression starting from day 7.

In particular gene expression results have shown a time dependent development of the fully developed hair bulb model that better mimic intact dermal papilla in comparison with traditional 2D culture and the temporal evolution of our in vitro model compared to in vivo hair follicle cycle. This project is currently under development in order to confirm these preliminary results, to better characterize the model for its dynamic evolution during an extended culture time and finally to investigate the rising of the regressive phase (catagen-like).

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