Hair follicle bulb developed as 3D scaffold free microtissue

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The hair follicle is a self-renewing "mini-organ" which undergoes to continuous cycles of growth and regression. 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. The hanging drop technology was applied to develop a DPF-ORSK co-culture in order to create a scaffold free "proto-follicle".

A multi-parametric approach was adopted in order to characterise the model and to have a comprehensive view of its evolution during culture time.
Viability by quantitative measure of cellular ATP and H&E staining coupled with immunohistochemistry and immunofluorescence on selected biomarkers served to monitor the presence and maintenance of type-specific features of the two cell populations (ck6, collagen IV).

To verify the feature of 3D proto-hair model compared to 2D culture, the expression of key genes by RT-qPCR (Taqman technology) during the time course were evaluated in comparison with HHDPC in 2D.
Two aspects of hair follicle physiology have been considered: a) metabolic activity and growth by quantifying genes that promotes the active phases of hair growth as BMP2 and FGF7 b) hair follicle structure evaluating the interaction between the mesenchymal cells of the dermal papilla and the epithelial cells of the outer root sheath LAMC3.

The results of gene expression has shown a time dependent development of the fully developed hair papilla model suggesting a anagen-like phase between 3 and 6 days cultivation in the plate and a probable catagen-like phase after 7 days in a co-culture model where the 2 cellular compartments appear well differentiated.