A Regressive 3D Scaffold-free Micro Hair Follicle (μHF) to Assess FGF18 Peptide Mimetics

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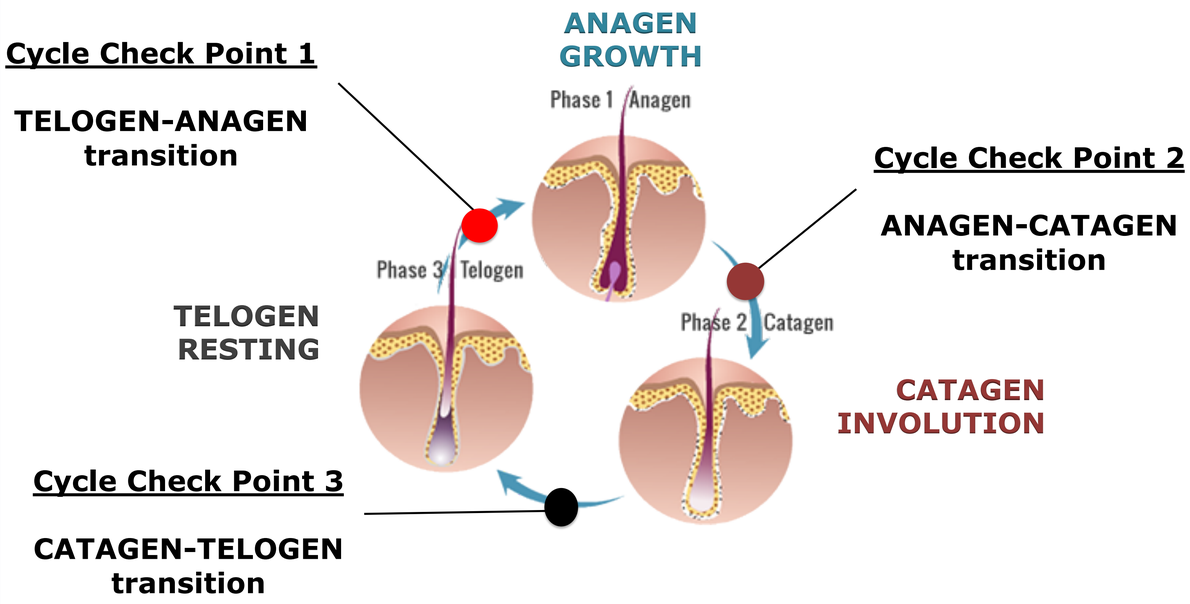
In memory of our friend and collegue
Marie Alice Dibon (Lyon February .1966 - Paris April .2019)

Introduction

The hair follicle (HF) is a self-renewing "mini-organ" which undergoes to continuous cycles of growth and regression, following a precise scheme in which a complex and fine-tuned interaction of signals induces deep metabolic and morphologic changes. The HF cycle is divided in an active growth phase (anagen), a regressive phase (catagen) and a final quiescence state (telogen) (1-2).

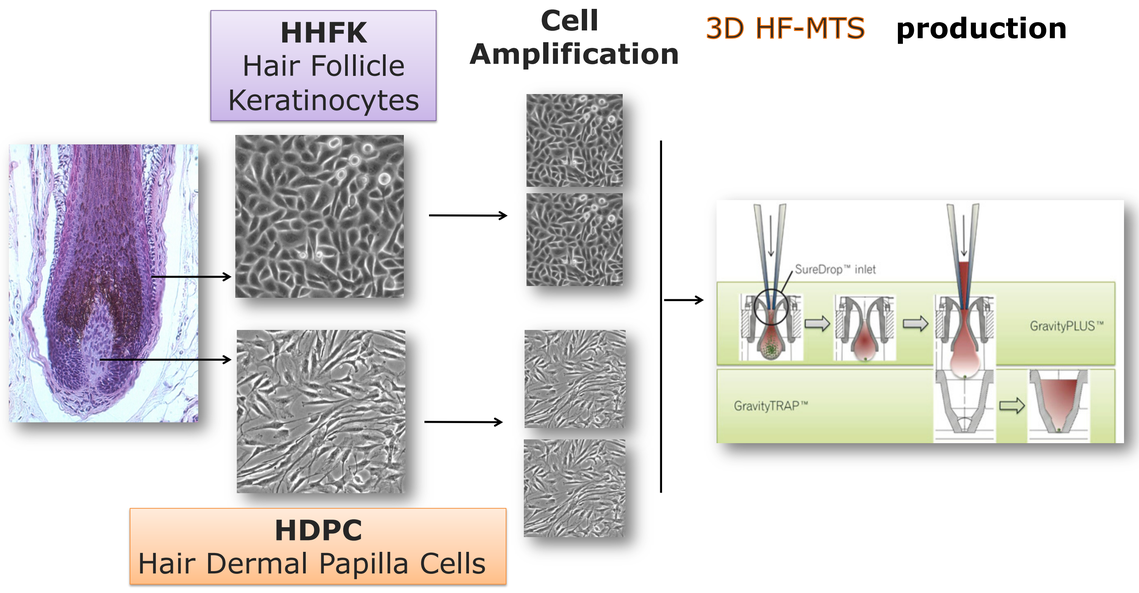


HF cycle as a biological clock. *Source: ShutterStock.com*

According to the HF cycle, **hair growth block** can be achieved by:
- **Anticipating Catagen Involution Phase**
- Maintaining the resting Phase Telogen

MicroHF DEVELOPMENT

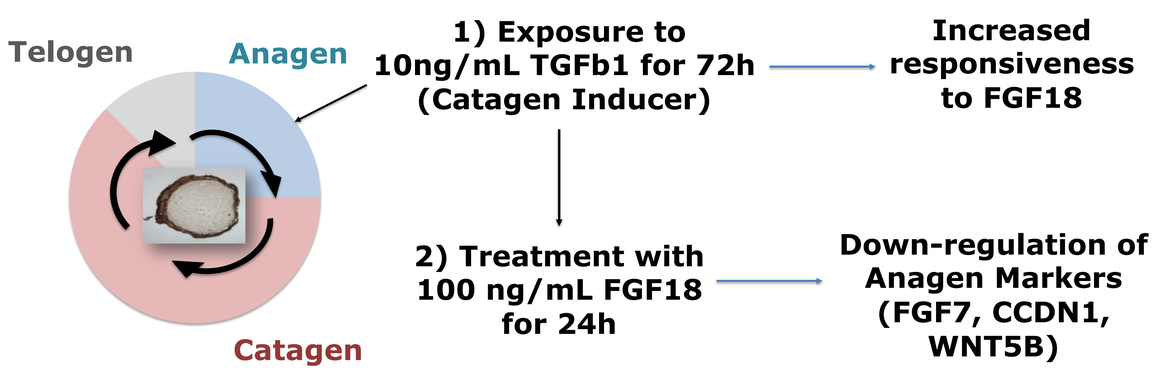
The μHF model was developed using cells from human hair follicle. After cultivation of Hair Follicle Keratinocytes (HHFK) and Hair Dermal Papilla Cells (HDPC) in monolayer, cells have been co-coltured following an optimized procedure based on hanging drop method and using patented plates (InSphero AG, Switzerland).



The obtained 3D scaffold free μHF are metabolically active, over-express the most relevant anagen biomarkers and can be considered in an anagen–like phase. As *in vivo,* μHF cycle is unidirectional but can be pushed toward late anagen and in defined conditions to catagen-like state.

Experimental design

In order to induce a catagen-like state (regression), μHF were exposed to TGFb1 and subsequently treated with Fibroblast Growth Factor 18 (FGF18) to further reduce anagen biomarkers expression (3). FGF18 is expressed during telogen in epithelial cells and exerts an inhibitory action on hair growth maintaining the HF in a quiescent state and avoiding the HF cycle re-start.



Objective:

The aim of the study was to establish a regressive HF *in vitro* model to elucidate the molecular mechanism underlying the HF quiescence state and develop tools for the screening of active compounds.

μHF GENE EXPRESSION CHARACTERIZATION

After exposure to TGFβ-1 the μHF has shown a significant down regulation of FGF7 gene expression and up-regulation of WNT5B (probably connected to epithelial re-modelling as it occurs in catagen).

After 24h treatment with FGF18, the regressive μHF has shown an increased down-regulation of FGF7 and CCDN1. WNT5B gene was also found to be down regulated after FGF18 exposure suggesting follicle quiescence and absence of epithelial compartment.

	Gene expression (RQ) in mHF	
	After exposure to TGFb1 (72h)	After exposure to TGFb1 (72h) and FGF18 (24h)
FGF7	0,42	0,06
CCDN1	0,70	0,36
WNT5B	3,18	0,72

Significant **over expression for RQ> 2; down regulation for RQ<0.5**

FGF18-LIKE PEPTIDE SCREENING

A **series of peptides** have been evaluated **at concentrations ranging from 1 to 100 ng/ml** after concentration selection in a preliminary cytotoxicity assay. Among 13 different **peptides**, 4 (at the moment under patent application by Rodan & Fields**) have shown a different efficacy in mimicking the effect of FGF18**

Table I. Effect of peptides (coded P1-P13) on the expression of proliferation/anagen markers in order to mimic a FGF18-like effect and promoting telogen phase/retarding anagen.

CODE	ng/ml	Potential effect on telogen phase induction and hair growth inhibition		
		CCDN1	FGF7	WNT5b
P1	100	+	+	+
P2	100	+	+	++
P3	100	no	+	no
P4	100	+	+	+
P5	100	+	+	+
P6	100	++	++	++
P7	100	+	++	++
P8	100	+	++	+
P9	100	no	+	no
P10	10	no	+	no
P11	1	no	no	no
P12	10	no	no	no
P13	1	+	+	+

(++) Higher effect in comparison with FGF18 100 ng/ml.
(+) Effect comparable to FGF18 100 ng/ml.

Conclusions

- A **regressive μHF model** has been developed based on exposure to TGFβ1.
- Exposure to FGF18 reduced proliferation and the expression of anagen genes related to hair growth (FGF7, CCDN1, WNT5B) mimicking a **telogen-like resting state.**
- This resting μHF model has been used to screen putative FGF18-like peptides for their ability to maintain quiescence.
- The screening results have shown a **different peptide efficacy according to their chemical structure and dose**.

References

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