

Investigating the effect of a test compound on hair follicle growth and anagen-catagen conversion

Question addressed: Does compound 'X' promote hair follicle elongation (hair shaft production) *ex vivo*?
 Does compound 'X' maintain hair follicles longer in anagen *ex vivo*?

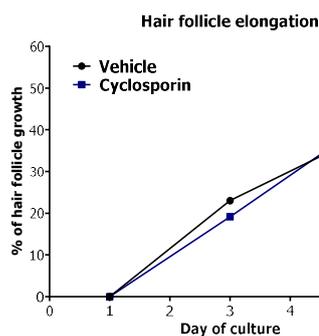
ML approach: Microdissected human scalp hair follicles harvested from at least 2 healthy donors, which spontaneously develop into a catagen-like state when organ cultured. Selected readout parameters can be evaluated in the entire hair follicle or selected compartments (incl. using laser capture microdissection) and quantified using various techniques, e.g. analysis of the culture medium, immunohistology and quantitative (immuno-) histomorphometry, qRT-PCR, *in situ* zymography, *in situ* hybridization, and microarray.

Possible claims: Compound 'X' stimulates hair shaft production *ex vivo*,
 Compound 'X' inhibits catagen development *ex vivo*

Case study: Cyclosporin maintains anagen *ex vivo*

→ Cyclosporin is well-known for inducing hair growth *in vivo*

1. Cyclosporin maintains the normal rate of hair elongation *ex vivo*



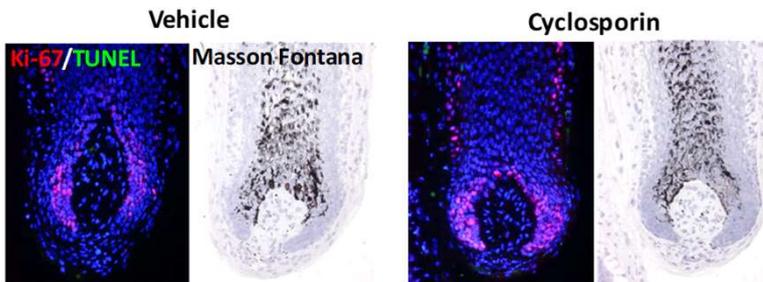
Amputated microdissected hair follicle at day 0, after isolation.



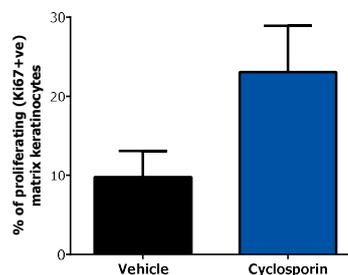
Amputated microdissected hair follicle at day 6 of organ culture: Note the newly formed hair shaft and outer root sheath

Data from 2 experiments, Mean, n=16-17 HF from 2 donors.

2. Cyclosporin maintains hair matrix keratinocyte proliferation *ex vivo*



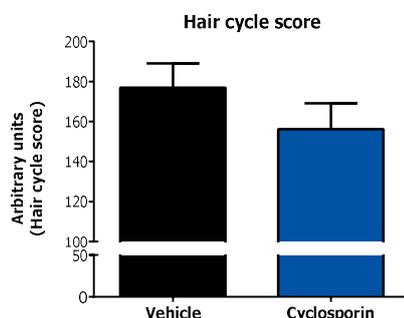
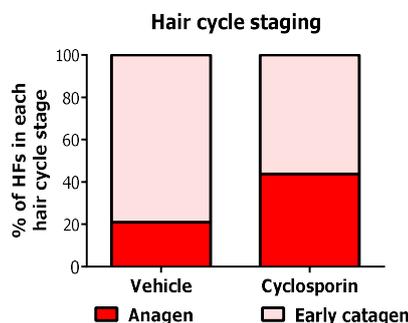
% of proliferative hair matrix keratinocytes



Pooled data from two independent experiments. Mean±SEM n=13-16HFs analysed/group from two donors.

Ki-67: marker for proliferation. TUNEL: marker for apoptosis. Masson Fontana: histochemical staining revealing melanin content

2. Cyclosporin maintains hair follicles longer in anagen *ex vivo*



Hair cycle staging and score analysed as previously published (Langan et al., Exp. Dermatol 2015)

Pooled data from two independent experiments. Mean or Mean±SEM n=13-16HFs analysed/group from two different donors.

➤ Additional markers available with possibility of establishing 'specific' customized markers