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A technique for more precise distinction between catagen and telogen human hair follicles ex-vivo

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28 Identifying human anagen hair follicles (HFs) *ex vivo* is readily accomplished by
29 stereomicroscopic analysis. However, to reliably distinguish other hair cycle stages, namely
30 late catagen and telogen, by stereomicroscopic analysis alone is difficult, and the “gold-
31 standard” remains histological analysis, which obviously precludes their use for *ex vivo* culture
32 (1,2). In this study, we sought to determine whether methylene blue (MB), a vital stain than
33 can be applied to living cells (3), helps to distinguish late catagen from telogen HFs *intravitaly*
34 for subsequent organ culture, thus greatly expanding translational preclinical research into
35 these as yet poorly investigated, but clinically important, human hair cycle stages.

36 Taking advantage of follicular unit (FU) hair transplantation methodology, when FUs are
37 grouped based on the number of HFs they contain (4), we recorded the number of anagen,
38 catagen and telogen follicles found in 800 FUs from 8 Caucasian male patients (100 FUs per
39 patient) undergoing a standardized FUE hair transplant procedure, with informed patient
40 consent. Since anagen VI follicles are easily identifiable (1), only those FUs that contained
41 catagen and/or telogen HFs were further microdissected, photographed, and immersed in
42 0.02% MB solution dissolved in saline (~5 minutes), followed by fixation and subsequent
43 evaluation.

44 As shown in Fig. 1, intravital MB staining greatly enhanced anatomical HF structures that could
45 be visualized by light microscopy alone and permitted correct hair cycle stage classification
46 using accepted, well-defined morphological criteria (2) such as the identification of a
47 prominent epithelial strand (Fig. 1a), a key feature of late catagen HFs, which is absent in
48 telogen HFs. Correct hair cycle stage classification by this method was confirmed by
49 Ki67/TUNEL immunofluorescence microscopy (Fig. 1d).

50 Importantly, MB staining enabled correct identification of the hair stage in 95.63% of cases,
51 compared to 72.02% in non-MB stained HFs. Thus, this simple, economical, and fast technique
52 constitutes a significant methodological advance in human hair research, since it greatly

53 facilitates *ex vivo* research on human catagen and telogen HFs without having to resort to
54 histology.

55 Our analyses revealed a higher percentage of catagen than telogen HFs in all patients (89%
56 anagen, 6.7% catagen, and 3.6% telogen). This supports the previous proposal that the
57 percentage of scalp telogen HFs has been overestimated (2), and questions the accepted
58 'standard' percentages of 80-89% anagen, 10-20% telogen and 1-5% catagen in the literature
59 based on transversal histologic sections (5) and/or (photo-trichograms), neither of which can
60 definitively distinguish between late catagen and telogen HFs. Although in our study the HFs
61 were from patients with androgenetic alopecia (AGA) and the ratio of anagen:catagen:telogen
62 may differ in comparison to non-AGA individuals, we believe that our data are unlikely to
63 reflect sampling bias, as HFs were harvested from occipital scalp, generally unaffected by AGA.
64 We propose that hair stage distribution in healthy human scalp needs a more systematic re-
65 evaluation, including comparative studies with histological sections. This is important when
66 assessing candidate hair growth-modulatory agents, since minor shifts in the percentage of
67 telogen or catagen HFs can result in major changes in the degree of visible effluvium.

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69 **REFERENCES**

70

71 1. Kloepper JE, Sugawara K, Al-Nuaimi Y, Gáspár E, van Beek N, Paus R. Methods in hair
72 research: how to objectively distinguish between anagen and catagen in human hair follicle
73 organ culture. *Exp Dermatol* 2010;19 (3):305–12.

74 2. Oh JW, Kloepper J, Langan EA, Kim Y, Yeo J, Kim MJ, et al. A guide to studying human hair in
75 vivo . *J Invest Dermatol* 2016; 136(1): 34–44.

76 3. Clifton J, Leikin JB. Methylene blue. *Am J Ther.* 2003;10(4):289–91.

77 4. Vogel JE, Jimenez F, Cole J, Keene S , Harris J, Barrera A, et al. Hair restoration surgery: the
78 state of the art. *Aesthet Surg J.* 2013; 33(1): 128–51.

79 5. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens
80 in male pattern androgenetic alopecia. *J Am Acad Dermatol.* 1993; 28 (5 Pt 1):755–63.

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82 **FIGURE LEGENDS**

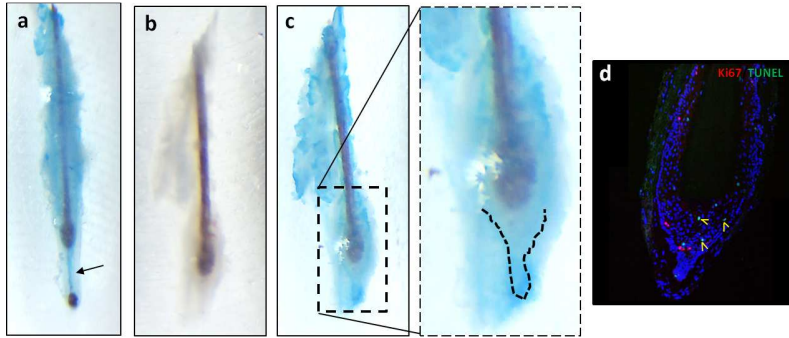
83 **Figure 1. Macroscopic analysis of hair follicles isolated from follicular units (FU) is more**
84 **definitive after Methylene Blue staining.** (a) HF in the late catagen stage with the epithelial
85 strand (arrow) clearly visible after MB (0.02%) staining. (b) A HF which cannot be clearly
86 identified under the stereomicroscope as either late catagen or telogen, followed by MB
87 staining (c) which highlights the small remaining epithelial strand (dotted line) which allows us
88 to identify it as late catagen. (d) Ki67/TUNEL confirms this is a late catagen HF, with the
89 presence of several apoptotic, TUNEL positive cells (arrowheads).

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