In vivo assessments of hair greying treatments

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Hair colour has long been a symbol of youth and health. The progressive loss of pigmentation in the hair shaft (hair greying or canities) is one of the most obvious signs of ageing, being not only an aesthetic concern but also a social and psychological challenge for many individuals.

These perceptions can result in decreased self-esteem, social withdrawal, reduced professional opportunities, and more generally in a decline in the quality of life. It has been reported that worldwide 6–23% of people have 50% grey hair by 50 years of age.

Onset of greying typically begins in the mid-30s for Caucasians, the late-30s for Asians, and the mid-40s for Africans. When the onset occurs earlier, canities are referred as premature. In men, grey hair typically begins at the temples and sideburns, then spreads to the vertex and lastly to the occiput whereas in women greying develops at the boundaries of the scalp and moves towards the vertex.[1]

With the increasing life expectancy, cosmetic demands for maintaining a youthful appearance are also rising. Despite this, research on canities has remained relatively limited. The purpose of this article is therefore to shed light on this subject.

Etiology of grey hair

Keratin, the main constituent of the hair, is colourless. The colour of hair comes from melanin pigments granules located in the cortex. As for the skin, melanin is produced by melanocytes within organelles called melanosomes that are transferred to keratinocytes of the hair shaft. The ratio of black-brown eumelanin to yellow-reddish pheomelanin, the quantity, and the distribution of the pigments determine the final hair colour.

Hair follicle structure is divided by the 'line of Auber', which separates the lower undifferentiated cell region from the upper differentiated region that forms the inner sheath and hair (Figure 1).

Cells move from the matrix to the upper bulb, elongate vertically, and some still show mitotic activity, though insufficient for significant hair growth. In pigmented hair, the pigmentary unit is a black structure at the dermal papilla tip above Auber's line, containing melanogenically-active melanocytes.

Below the line, only unpigmented and undifferentiated melanocyte stem cells are typically found. In grey hair, the pigmentary unit becomes less defined, with fewer, rounded melanocytes and lightly pigmented oligodendritic melanocytes detectable below Auber's line.

Normal melanogenesis of human hair is cyclical and occurs only during anagen III-VI phases when the hair follicle generates a hair shaft. Without a good coordination between the proliferation and differentiation of keratinocytes and melanocytes during this period of the hair cycle, a normal pigmentation cannot be achieved.

Melanosomes loaded with melanin pigments are transferred from melanocytes into keratinocytes of the hair shaft during these phases. As a consequence, it means that greying happens during the anagen phase (and can only be reversed in this phase too).

Hair progressively appears grey because there is less and less melanin in the hair shaft keratinocytes. There are several possible causes for this melanin decrease. Firstly, this pigment loss results from a reduction in the number of melanogenically active melanocytes and a depletion of melanocyte stem cells in grey anagen hair follicles.

Secondly, greying may also involve defective melanosomal transfer to cortical keratinocytes. Melanosomes may also be removed by autophagy. Thirdly, the decrease of melanin synthesis is due to a decrease in tyrosinase activity. Indeed, true grey hair shows reduced tyrosinase activity, while white hair bulbs show none.[2]

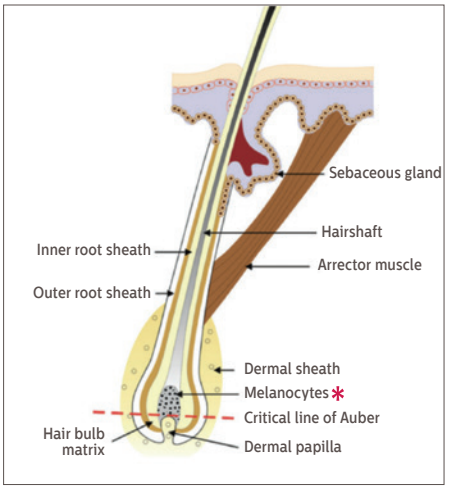

Figure 1: Pigmentary unit in relation to the dermal papillae and the line of Auber

A large body of evidence supports the free radical theory as a major cause of greying. Indeed, oxidative damage is common to the above processes. Melanogenesis generates high oxidative stress via the hydroxylation of tyrosine

and the oxidation of dihydroxyphenylalanine (DOPA) to melanin. Byproduct reactive oxygen species (ROS) induced during melanin biosynthesis have a detrimental effect upon both melanocytes and upon melanosomes transfer into keratinocytes.

At the same time, with ageing, there is a decline in the ROS scavenging and damage repairs abilities which are supposed to counteract oxidative stress. Risk factors associated with canities include some auto-immune disorders but also external factors such as nutritional deficiencies. The roles of genes in human hair greying is still poorly understood but heredity is likely to play a role.[3,4]

Hiding or reversing hair greying

The English writer P.G. Wodehouse wrote 'There is only one cure for grey hair. It was invented by a Frenchman. It is called the guillotine'. As one of the author of this paper is French, we feel obliged to disagree with this very strong statement. At least partially, because it is true that hair greying was believed during a long time to be an irreversible process.

More seriously, because of this belief in the impossibility to reverse the natural course of hair greying, hair dyes were – and mainly are still – the only solution to bring back the native colour of hair.[5] Natural dyes such as henna were a traditional option although henna typically provides reddish or orange hues on grey hair.

Other natural dyes can offer brown or black hues. Hair dyes come in various types, each with different characteristics and purposes. Permanent hair dye contains chemicals that penetrate the hair shaft and are then oxidized by hydrogen peroxide to activate the colour change.

The advantage of permanent dyes lies in the long-lasting effect and its resistance to normal hair washing. Semi-permanent hair dyes are small molecules that penetrate the cuticle, adding colour to the outer layer of the hair, but gradually fading until disappearance after 4 to 12 shampoos.

Finally, temporary hair dyes consist of large complex organic structures that do not penetrate the cuticle, therefore providing a short-term colour change which often lasts until the next shampoo only. Semi-permanent and temporary dyes are gentler for the scalp and hair but their camouflage effect is inferior to that of permanent colourants.

In the medical field, a growing number of grey hair repigmentation cases have been reported following diverse pharmacological interventions, thus challenging the notion of hair greying irreversibility.[1,2] Many case reports originated from the observation of 'side effects' of anti-inflammatory, chemotherapy treatments for instance.

Due to the nature of these molecules, their cosmetic use for darkening hair is excluded but understanding their mechanisms of actions could well open, one day, the way to new hair repigmenting actives. Theoretically, hair greying could be prevented if ROS are adequately removed by effective antioxidants. So deciphering the susceptibility of melanocytes to oxidative stress might yield clues to possible treatments for the prevention and reversal of canities.

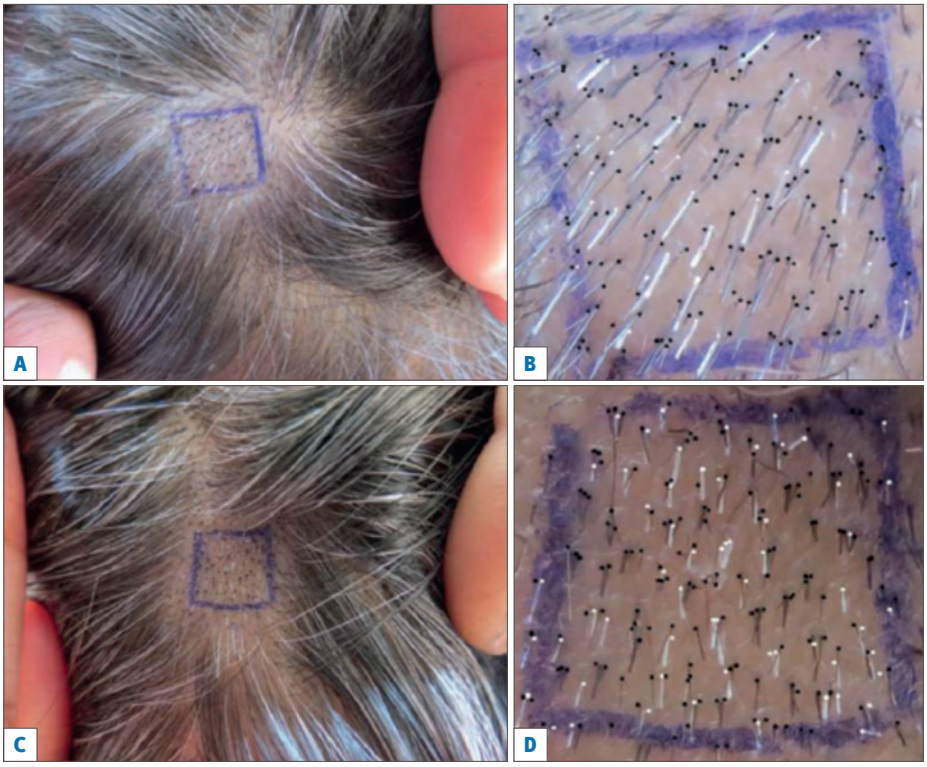

Figure 2: (A and C) Clinical photograph demonstrating a 1 cm^2 area and clipped to approximately 1 mm above the scalp surface (B and D). Magnified view of the photograph (white and black dots represent grey and black hair, respectively). Reproduced from Singal *et al.*

In recent years, an increasing number of anti-grey hair treatments aiming at preventing or reversing canities have entered the market. Many are based on herbal extracts or blends of vitamins, B12 being especially popular. As for today, there is limited scientific evidence that certain treatments can actually reverse hair greying. This lack of proof pleads for the need for rigorous evaluation of the efficacy of anti-canities interventions.

In vivo assessment of interventions in hair greying

Publications about substantiation of cosmetic and pharmacologic interventions for canities are very scarce. There is no method universally adopted by the personal care industry. Here we will present a selection of the most common available techniques and will discuss their main advantages and shortcomings.

The easiest method relies on self-assessment based on questionnaires. This is very simple, suffers from its subjectivity by definition, but has nevertheless some interest when performed on a population large enough since results are a reflection of the perception of consumers.

Slightly less subjective is self-grading where subjects rate different hair descriptors by using visual analogue scales (VAS) for example. It typically consists in marking a point on a line with endpoints defining the extremes, from 1 (all hair pigmented) to 10 (all hair white), for instance.

The distance from the minimum to the mark provides a quantitative score. VAS is valued for its ease to use, making it a popular choice in cosmetic/dermatologic research settings. When the grading is done by trained evaluators or by dermatologists, a more objective assessment can be achieved.

Different specific scales have been used. Recently a study was based on the assessment of the proportion of grey hair (scale from 0% to 100%) determined by the blind scoring of photographs taken at baseline and Tn.[6] As an attempt to standardize and achieve objective and reproducible means of assessing the severity of the greying of hair, the Greying Severity Score (GSS) has been proposed.[7]

The GSS requires the acquisition of photographs of five scalp areas of 1 cm^2 taken on the vertex, right and left temporal, frontal and occipital regions. A score is then allocated to each region based on the computer-assisted counting the numbers of grey and coloured hairs by experts (Figure 2).

Finally a cumulated score is calculated. The main limitation of this scoring system, according to the authors themselves, is its time-consuming nature. Although a score if the final outcome of the above GSS, the method is actually a combination of digital photography/image processing and clinical scoring. This leads us naturally to the more objective and quantitative instrumental and photographic methods.

What comes to mind first when we are interested in the instrumental measurement of hair colour are chromametry and spectrophotometry. Indeed, many laboratories have used these gold standard techniques, either for fundamental studies of the diversity of the natural colour of the human male and

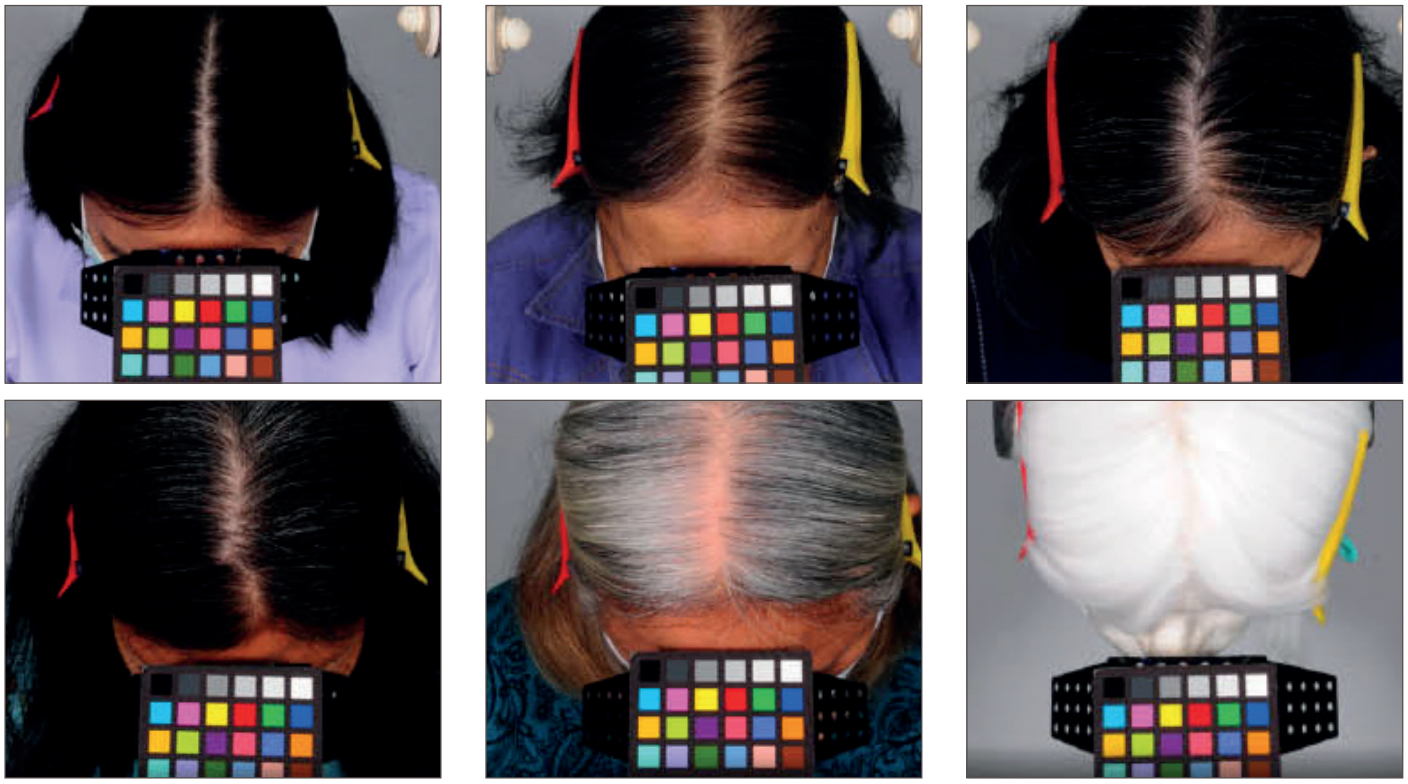

Figure 3: Subjects with exhibiting increasing degrees of hair greying for determining the linearity of grey hair quantification (on the right: virgin human white hair wig)

female hair from 23 regions of the world[8], or for cosmetic tests.

Colour measurements using these instruments are commonly expressed using the CIE L*a*b* space because of its closeness to the human perception of colours. Luminance L* which measures the lightness from 0 (black) to 100 (white) and b* are the variables of interest (a* is interesting only when blonde-red hair are investigated).

Measurements are sometimes performed directly on the head of the subjects, provided that a reliable repositioning system exists to ensure that measurements are always done on the same location at each kinetics. Yet it is more usual to work on strands of hairs collected by cutting and put in customized sample holders.

Indeed, many geometric and physical factors may impact the measurements. Hair fibres are covered by layers of cuticles which influence the reflection of illuminating light. They also can have different shapes and alignment. Hair fibres heterochromia (mixed colours) add one more level of difficulty. And finally, hair styling can vary during studies.

All these heterogeneities and the fact that chromameter/spectrophotometer probes have small apertures, make it relatively difficult to obtain reproducible and representative data on hair colour. Digital photography is therefore an attractive alternative and has been the most favoured technique these last years.

The main advantage is the possibility to obtain in one single shot an image of a large area. Firstly, this allows one to 'dilute' the sources of heterogeneities mentioned previously. Secondly, RGB pixel values can be converted into the L*a*b* colour coordinates familiar to cosmetic scientists.

Several commercial imaging systems are usable for the accurate quantification of the proportion of grey hair based on colour analysis. In our laboratory, we have decided to develop a method consisting in acquiring photographs of the mid-scalp area of subjects with the handheld Antera 3D® camera (Miravex, Ireland).

The associated software can determine the L*a*b* values. The validation of the technique has been previously reported.[9] Briefly, part of the validation consisted in checking the reproducibility, repeatability and linearity of the method. The latter was done on subjects with different and increasing degrees of greying (Figure 3) and also by introducing various quantities of white hairs to natural ones.

Conclusion

Hair greying is a natural phenomenon and almost inevitable for all the persons who have the chance to live long enough. For a long time, camouflage with dyes was the only possible strategy to conceal canities. It is still used as a primary treatment.

Now, reversing greying thanks to cosmetic or pharmacological interventions seems more approachable. The effectiveness of these new treatments will need to be proven. *In vitro* and *ex vivo* methods exist for the assessment of hair greying. In this paper, we have focused on *in vivo* evaluation and quantification methods because of their direct relevance to the substantiation of claims, but the *in vitro* and *ex vivo* tools should neither be forgotten nor neglected.

Finally, we would like to end with some additional remarks about unpigmented hair. Grey and white hair differ from coloured hair not only by their hue. They exhibit a faster growth, are thicker, have faster water sorption and desorption. Hair fibres have different physical properties and lipids composition. Therefore, taking care of aged hair should not be restricted to the management of greying. **PC**

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