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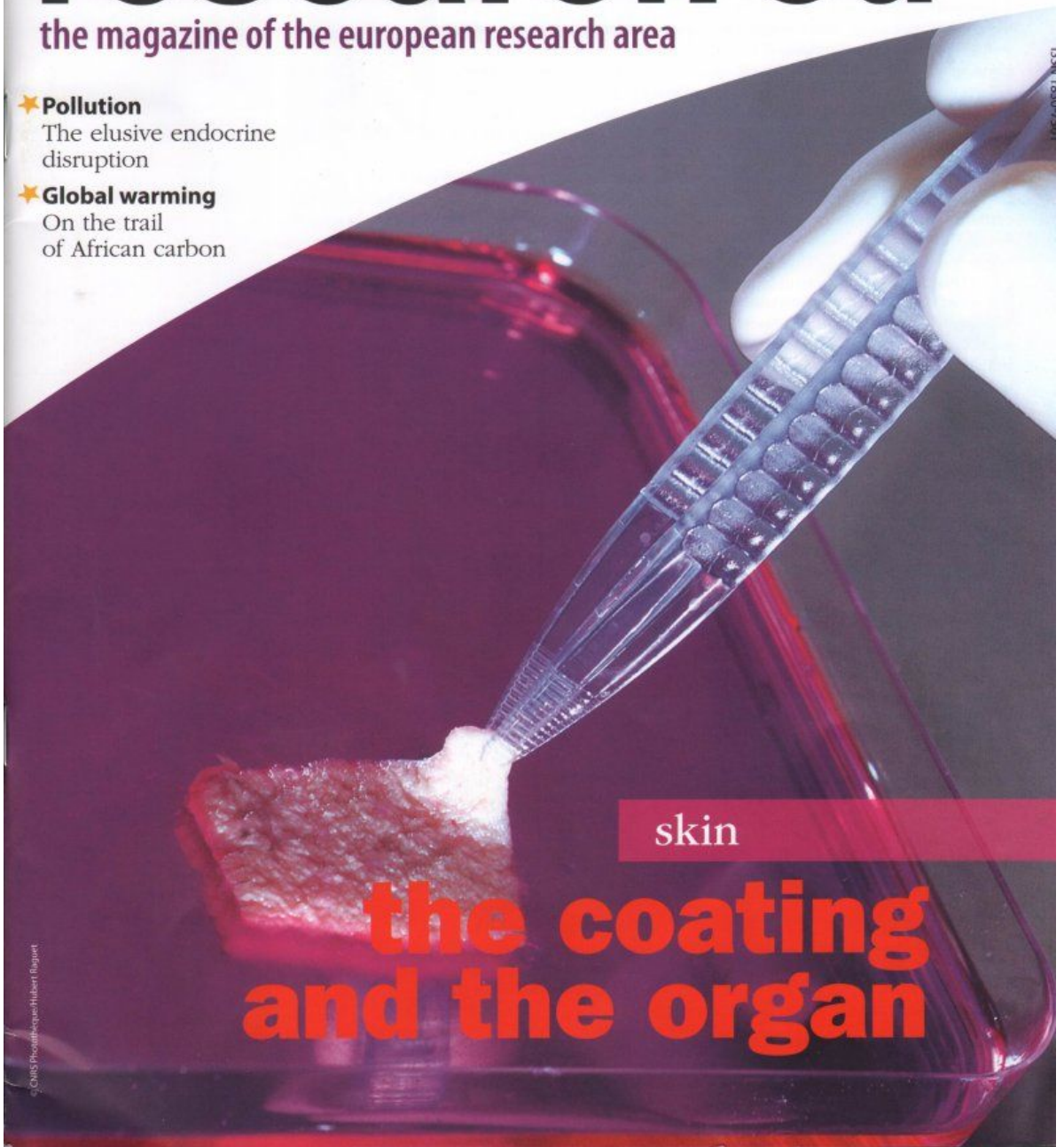
Pollution
The elusive endocrine disruption

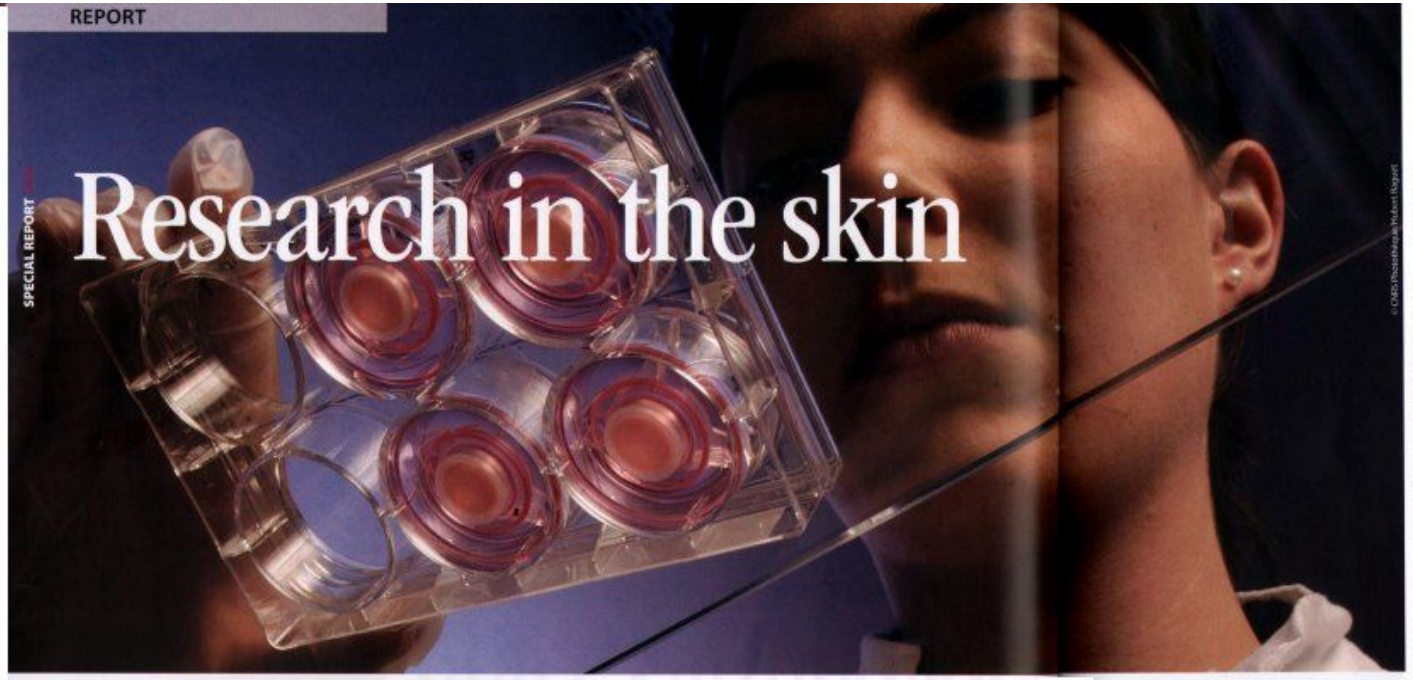
Global warming
On the trail of African carbon



skin

the coating and the organ

Research in the skin



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Application of a cosmetic product on a reconstructed skin cultured on an insert. This is used to test products without using animals.

In the star-spangled world of cosmetics, research is not exactly a centre-stage activity. Yet companies sometimes invest considerable sums of money in the scientific expertise needed for developing certain products. We visit the laboratories of French cosmetic giant L'Oréal.

Since 2000, Europe has had a tough time getting business and governments to sign up to the Lisbon Strategy, which would have the European Union spend 3% of GDP on R&D. The current objective is to combine public and private efforts to achieve this by 2010. Whilst Europe's private sector is generally somewhat hesitant, certain enterprises have long allocated substantial resources to research. One such company is L'Oréal, which in 2007 earmarked €560 million, or 3.45% of annual sales for this purpose. According to the company, a third of this budget is spent on 'advanced' research into the basic mechanisms of skin and hair.

Truer than life

There is one part of our body that represents nearly one-sixth of our weight and has the fantastic ability to rebuild itself naturally when damaged. This is the high-tech protective envelope that we call our skin. For a cosmetics company, clearly understanding the reconstruction of natural tissues is a major advantage when testing the harmlessness of its products. But quite unsuspected medical applications derive from this as well. Treatment of severe burns is a good example. For those injuries where the surface area of a deep burn is more than 50%, self-grafting of healthy skin to cover the wound is no longer possible and a graft of cultured skin becomes essential. Reconstructed tissue is also necessary for developing new skin disease treatments because it permits better understanding of the physiology of healthy and pathological dermis.

Which cells should be selected? How do we control their growth? On what substratum? These are all questions researchers have been trying to answer for 30 years or more, in order to develop a *skin-equivalent* model. "Initially, cells are taken from tiny pieces of skin removed surgically from healthy volunteer patients. These are then inserted in a matrix of collagen and elastic fibres, itself synthesised by fibroblasts," explains Marcelle Régnier, head of the skin reconstruction laboratory. "To produce *in vitro* models from a skin sample, we detach the epidermis from the dermis and separate out the epidermal cells to obtain a cellular suspension. This is placed on a semi-solid support and immersed for a few days in a proliferation liquid. After this the recombinant support-cell is placed in contact with the air, which causes the stratification of the sample. After about 10 days, an epidermis forms with all the normal cell layers, including the protective corneal layer.

A skin of choice

Their desire for optimal use of skin reconstruction expertise led L'Oréal to approach the Hôpital Percy (FR). "Improving the results of grafts on third-degree burns was our first objective," says cell therapy unit head Jean-Jacques Lataillade. "What interested us was access to the culture protocol L'Oréal uses. This enabled us, in record time, to seed keratinocytes on a fibrin film and obtain, in just over a week, a strip of epidermis that could be transplanted onto the burn.

"In the short term, we are planning to use stem cells to improve the adhesion of the graft and the treatment of the underlying dermis," he concludes. Marcelle Régnier goes even further: "We hope soon to be able to set up a bank of deep-frozen skins corresponding to the different existing phenotypes. Controlling skin colour is no longer a major concern. In the very latest model of skin equivalent 'art', researchers can control the number of melanocyte seeds, allowing them to define the skin colour in response to the needs of different populations. The commercial interest of this advance is obvious. For grafts, this technique also makes it possible to further reduce the difference between the graft and the patient's own healthy skin".

Allergy problems have not been forgotten either. Langerhans cells, our body's skin allergy 'detection agents', have also been incorporated into the model. This crucial advance, which was the result of a collaboration financed by FP6, today permits live-scale testing of cutaneous reactions to a cosmetic or pharmaceutical ointment. "This was a little more complicated," Marcelle Régnier admits. "Unlike keratinocytes or melanocytes, these cells do not multiply *in vitro*. We therefore use their precursors, which are present in the bloodstream and in the blood of the umbilical cord. After multiplication in sufficient quantities, these precursors are included in the epidermal cell suspensions to obtain a skin model which integrates Langerhans cells."

Sun and skin: no love lost

It is a well known fact that skin and sun do not mix well. Beyond the 'healthy look' of a tanned body, it is a proven fact that chronic exposure to the sun, even in low doses, provokes a premature ageing of the skin. "As a result, nascent skin cancers on the face or hands are extremely common among the elderly," confirms Françoise Bernerd, who runs the photoprotection laboratory.

The more energy in a sun's ray, the weaker its penetrating power. In the case of UVBs, their energy makes them particularly absorbable by the nucleic acids of the epidermis, and hence by the DNA of those cells. This absorption induces distortions which provoke direct lesions. In healthy individuals, these lesions are located, removed and repaired by a specialised enzyme system, known as *Nucleotide Excision Repair – NER*. "Proof if ever it were needed that nature does things well," says Françoise Bernerd, head of the photoprotection laboratory, "is that a protein, P53, blocks the cell cycle to prevent duplication of the failing cell until the rectification is complete."

In the longer term, however, frequent exposure produces successive lesions, increasing the risks of errors which cause light-induced cancers. And contrary to what one might think, these lesions occur long before a sunburn appears.

A leg-up for XPs

In very rare cases, children are hypersensitive to the solar spectre, caused by a mutation making enzymes unable to carry out these repairs. These young patients, sometimes called 'moon children', are condemned to hide by every possible means from the sun's rays. Suffering from *xeroderma pigmentosum – XP* – they present 2000-4000 times more precancerous lesions than the normal population, in a dramatically precocious fashion.

In a collaborative project with the *Institut de cancérologie Gustave Roussy* (FR), models of reconstructed skin were produced from the cells of sick children. Thanks to pooling their skills, four years of research were sufficient to develop an ex vivo model of skin whose UV response matches that of XP children. "Culturing cells taken from these patients was a very delicate task, as they reproduce much less efficiently," Françoise Bernerd stresses. "But these difficulties have enabled us to detect many anomalies linked to the disease and better understand how it functions."

Thierry Magnaldo, research director at the *Institut Gustave Roussy*, speaks of recent advances: "One of our doctoral students has succeeded in correcting the defective genes of a piece of reconstructed XP skin by replacing them with a healthy gene. These pieces of corrected tissue have been grafted onto mice in order to observe the long-term behaviour of the graft. As results to date have shown real resistance to UV, we are planning in the future to offer patients the choice of a graft of genetically corrected epidermis to replace excised skin tumours. ●●●

●●● For Thierry Magnaldo, the speed with which these results have been obtained is due, beyond any doubt, to the cooperation between the academic unit and the private firm. "The complementarity of our two laboratories was obvious. We had the expertise in DNA repair mechanisms and had devised a technique for identifying the seven genes at the origin of the disease. L'Oréal provided us with their knowledge of cutaneous responses to UV, and in particular of reconstructed skins."

The interest for L'Oréal in turn is to understand the harmful effects of UV over a shorter period, as these patients simply develop at an abnormally high rate a pathology which anyone regularly exposed to the sun is likely to present in the longer term. Developing hyperphotosensitive skins is also essential as it is difficult to imagine volunteers ready to submit to a high dose of UV just to verify the efficacy of new sun protections.

Hair follicles: the veil lifted

Studied under every 'scale', human hair has few secrets left for scientists. But the same is not true for the hair follicle, the organ which produces it. Bruno Bernard, who heads the hair follicle biology unit, looks back on the circumstances of its creation. "When this laboratory was created 16 years ago, our knowledge of the subject was almost zero. Given the work ahead of us, the teams had to be multi-disciplinary. I needed biochemists, molecular biologists, physicians... in short, a host of varied profiles which are today the strength of our unit. Since then, discoveries have come in rapid-fire succession with no shortage of promising and surprising results."

The hair follicle is a unique organ in the human body. First of all because, after dissection, it is the only organ to maintain active production and to continue to produce hair *in vitro*. Second, it degenerates cyclically: when a hair falls out, the entire organ disappears, to be renewed from adult stem cells. Lastly, it is the only organ for which these stem cells have been located and for which the gene expression profiles are fully known.

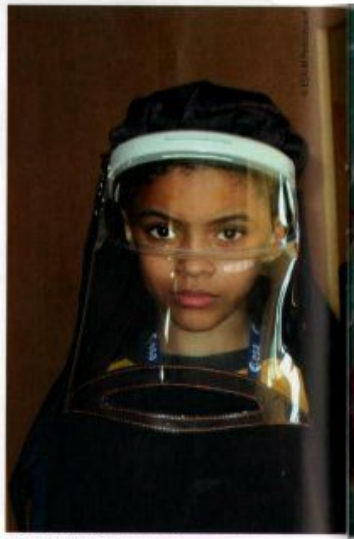
"The hair follicle is undoubtedly the reservoir par excellence of adult stem cells," says Bruno Bernard. Adult stem cells are no longer multipotent like embryonic stem cells. But under ad hoc culture conditions, they can be differentiated within a certain repertory which, in the case of the hair follicle, includes the nerves, bones, skin and, of course, hairs. "Imagine you have a nerve which is not doing its job. This is pretty annoying. All you would need to do is to dissect a few scalp hair follicles, extract from them the right cells and have them differentiated to produce a conductive shaft that can be grafted with no risk of rejection." This is not science fiction. The technology has already been successfully tested on mice at McGill University (CA).

Growth of hair follicles in vitro.

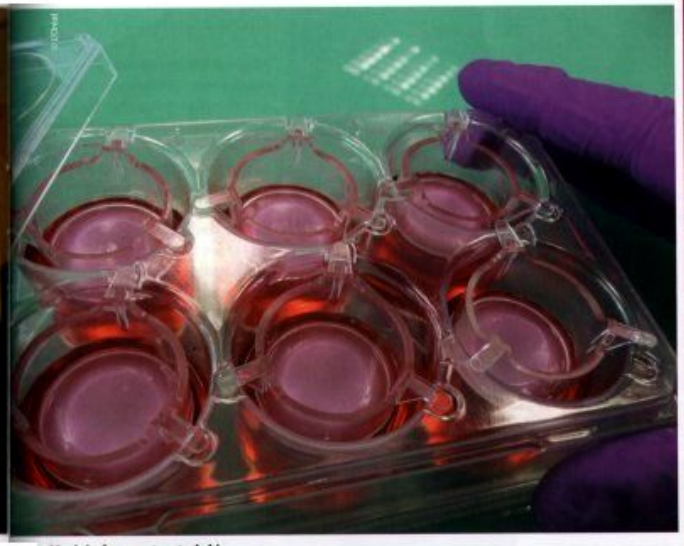
Greying revisited

The natural discolouring of hair is one problem cosmetics researchers are keen to resolve. One can easily imagine the success of an effective anti-greying product. But to understand how hair goes grey, one needs to know what gives hair its colour in the first place.

Early on, one of the research projects put its finger on the cause of greying. The melanin pigments are produced by the melanocytes of the hair follicle. Whilst at skin level melanocytes have a reduced basal activity which can be 'boosted' by UVs, the activity of the follicle melanocytes is permanently high. When hairs fall out, as they do every day, the pigment producing units disappear and are regenerated from a reservoir of stem melanocytes. But this stock gets low with the passage of time, provoking gradually the appearance of grey hair – insufficient melanocytes – and white hair – absence of melanocytes. At this stage, however, no one knew why this reservoir ran down.



This special **anti-UV garment** uses space technology from the ESA to enable child *Xeroderma Pigmentosum* patients to walk around safely in daylight. The head protection includes a transparent visor and the garment, worn under usual clothing, has a special lining - used on space craft to form a total barrier against UV rays. A special cooling circuit is available for hot weather situations.



Model of reconstructed skin.

Parallel with this, another research project in the same unit brought to light a strange coincidence. During their analysis of melanogenesis, scientists noticed that the skin melanocytes which express the *Tyrosinase-related protein 2* – TRP2 – do not disappear. The other way round, skin cultures showed that those that do not express TRP2 are condemned sooner or later. The most interesting fact is that it is precisely the capillary melanocytes which lack TRP2. In other words, TRP2 does not affect colouring in any way, but on the other hand playing instead a cytoprotector role, the anti-oxidant effects of which appear to preserve melanocytes from certain disappearance. Two solutions to greying seem here to open up: either reintroducing the synthesis of this protein into the follicle; or mimicking through another molecule.

A theory-busting protein...

TRP2 is officially listed as one of the key melanogenesis enzymes. At least that is what most human biology textbooks tell us. But how then do we explain its absence in the hair follicle whereas the hair this follicle produces is clearly coloured?

This question teased Bruno Bernard. "The announcement of the absence of TRP2 in the follicle shook the community, calling into question a long-accepted theory." But if the wrong role has been attributed to it, what then is its real function? "When this protein appears during embryogenesis, it was noticed that it accompanies the enzymatic chain associated with the synthesis of dopamine in its migration from the neural crest. We suspect that TRP2 in fact protects against the effects of this dopamine which, while useful to the brain, becomes harmful in skin melanocytes. The presence of TRP2 may well protect melanocytes in the epidermis and its absence may weaken the melanocytes of the hair follicle. This theory is still highly speculative, but we have a series of clues which lead us to believe that we have put our finger on something really hot," our researcher concludes with an unconcealed smile. ●

Marie-Françoise Lefèvre

A mini-glossary of skin

Keratin: a fibrous, non-water-soluble protein which gives the skin its impermeability and its outer protection.

Keratinocytes: major components of the epidermis, keratinocytes are found in superimposed strata and synthesise keratin.

Melanin: the main pigment protection against UV rays. The dissemination of this macromodule in keratinocytes gives the skin its colouring.

Melanocytes: cells responsible for producing melanin grains. A melanocyte can supply melanin to 35-40 keratinocytes.

Fibroblasts: produce the collagen fibres found in the dermis. Anchored up into the epidermis these give the skin its resistance to outside deformations.

UVA: segment of the solar spectrum with wavelengths between 320 and 400 nm. With their high penetration powers, these rays pass through windows or windscreens to reach the dermis, the deep layer of the skin. In the long term they provoke premature photoageing.

UVB: segment of the solar spectrum with wavelengths between 280 and 320 nm. Their lower penetration power limits their absorption to the epidermis, the outer layer of the skin, potentially producing UV-induced cancers.