

Stem cell approach 'makes aged skin young'

Rather than act on stem cell multiplication or proliferation, Codif Recherche et Nature is developing a new approach to improve the mobilisation and renewal capacities of ageing stem cells by giving them back the activity of their youth.

Moreover, as dermal and epidermal stem cells have a different morphology, role and function, the company has revolutionised the concept of skin stem cells by developing two dedicated active substances: Phycojuvenine for adult dermal stem cells and Phycosaccharide AI for adult epidermal stem cells.

Role of adult stem cells in skin ageing

Unlike stem cells, which are pluripotent and may generate all cell types, adult stem cells are more limited as they are already specialised according to the tissue in which they are produced. However, they do have the same cell renewal qualities as stem cells.

The adult stem cells are rare and specialised but are necessary for tissue regeneration throughout the body's life. Every day, skin integrity is maintained by dermal and epidermal adult stem cells which self-renew and generate daughter cells which then differentiate into fibroblasts or keratinocytes.

Despite the presence of these adult stem cells, the skin ages, sags, and wrinkles. It also heals increasingly slowly.

Skin ageing and slowed regeneration are due to the poor mobilisation of stem cells, and/or to a reduction in the number of stem cells capable of responding to renewal signals.¹

In both cases, these dysfunctions are due to an ageing of adult stem cells. With time, their renewal capacities fall and tissue regeneration slows down.¹

Whereas the first "stem cell" approaches consisted in the stimulation of their proliferation to activate tissue regeneration, recent studies show that the exhaustion of stocks of adult stem cells in the skin may occur following forced proliferative stress. Molecular mechanisms are therefore capable of

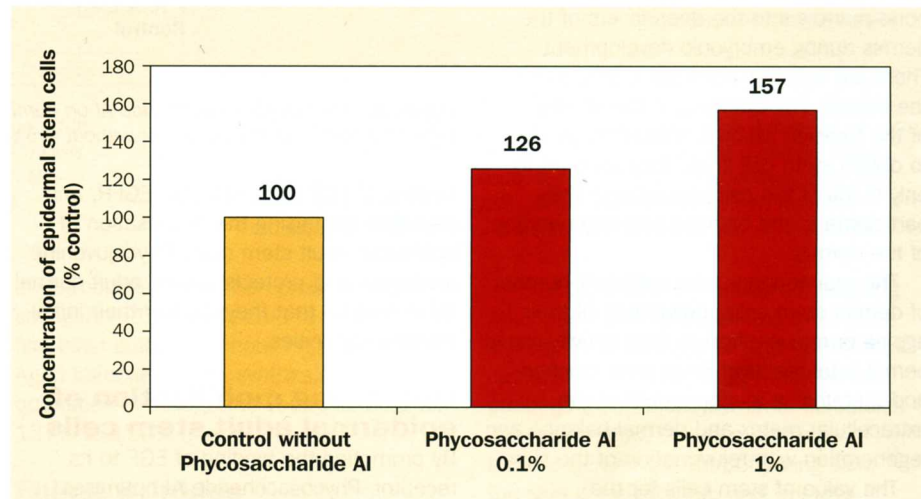


Figure 1: Effect of Phycosaccharide AI on the mobilisation of epidermal adult stem cells. Shown is the culture of epidermal stem cells with or without Phycosaccharide AI for 10 days. The number of cells is evaluated by optical density.

limiting the number of divisions of stem cells.^{2,3} Dermal and epidermal adult stem cells do not therefore have an infinite capacity for renewal.

Stimulation of regeneration of the dermis and epidermis does not therefore involve stimulation of adult stem cells, but a rejuvenation of their activity, or an optimisation of their mobilisation.

Epidermal adult stem cells

The first reservoir of adult stem cell of the epidermis is the bulge zone of hair follicles. Responsible for the integrity and balance of the epidermis, epidermal adult stem cells migrate along the dermo-

epidermal junction when damage occurs and multiply, differentiate, and regenerate injured tissue.

Their mobilisation is controlled by different factors including EGF (Endothelial Growth Factor) secreted by keratinocytes affected by the injury. By binding its receptor EGFR (Endothelial Growth Factor Receptor) located on the membrane of epidermal adult stem cells, EGF mobilises stem cells and activates tissue regeneration.

The studies of Kawada *et al*⁴ showed that some oligosaccharides, characterised by a specific mannuronic, and guluronic acid sequence, promote the assembly of

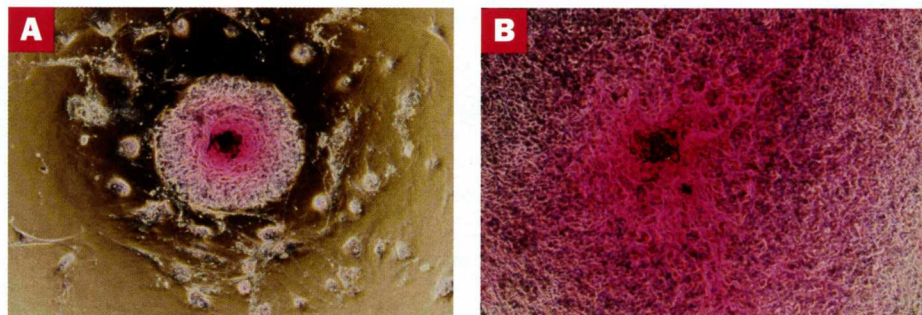


Figure 2: Effect of Phycosaccharide AI on the generation of colonies. Culture of epidermal stem cells on a fibroblast matrix without Phycosaccharide AI (2A) and with 0.5% Phycosaccharide AI (2B) for 12 days.

the two sub-units composing EGFR and thereby optimise the binding of EGF to its receptor.

Promoting the binding of EGF to EGFR may therefore make it possible to optimise the declining mobilisation of ageing adult epidermal stem cells and consequently epidermal regeneration.

Dermal adult stem cells

Like chondrocytes and osteoblasts, fibroblasts are mesenchymal cells. They are derived by differentiation of dermal adult stem cells which migrate from the bone marrow into the deep layers of the dermis during embryonic development. There are very few adult stem cells in the dermis. For example, in the dermis of the foreskin which is frequently used to obtain stem cell lines, they account for only 0.3% of the cell population.⁵ They participate in the balance and regeneration of the dermis.

The maintenance of a sufficient number of dermal stem cells, prevention of their ageing or rejuvenation of their activity would permit total reactivation of their function and therefore a reinforcement of the extracellular matrix and dermal balance and regeneration with rejuvenation of the skin.

The value of stem cells for the maintenance of skin integrity and therefore for the processes that combat ageing has been amply demonstrated.⁶ It is impossible, however, to directly interact on the multiplication processes of these stem cells, as this might initiate uncontrolled cell proliferation, or lead to the rapid exhaustion of their capacity for renewal.

Codif Recherche et Nature has studied the ageing parameters of adult stem cells, with the decline of their capacity for mobilisation and renewal. As already mentioned, two active substances specific to adult stem cells of the epidermis and dermis have been developed: Phycosaccharide AI and Phycojuvenine.

Phycosaccharide AI is an oligoalgininate composed of a mannuronic and guluronic acid sequence, capable of optimising the

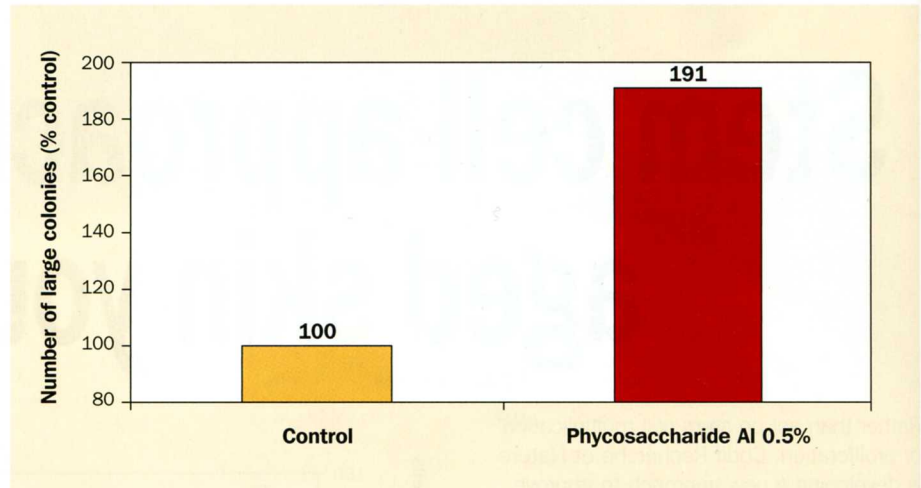


Figure 3: Effect of Phycosaccharide AI on numbers of large colonies. Culture of epidermal stem cells on a fibroblast matrix with or without 0.5% Phycosaccharide AI for 12 days.

binding of EGF to its receptor EGFR, and therefore optimising the mobilisation of epidermal adult stem cells. Phycojuvenine revitalises and protects ageing adult dermal stem cells so that they recover their initial renewal capacities.

Optimising mobilisation of epidermal adult stem cells

By promoting the binding of EGF to its receptor, Phycosaccharide AI optimises stem cell mobilisation. A larger number of epidermal adult stem cells therefore renew themselves so that their density increases: +57% compared to the control without Phycosaccharide AI (Fig. 1). Each mobilised adult stem cell which is renewed creates a cell colony. In the presence of Phycosaccharide AI the colonies are found to be larger (Fig. 2) and more numerous: +91% (Fig. 3).

These latter results suggest that tissue regeneration capacities will be much faster and more effective in the presence of Phycosaccharide AI.

Optimising epidermal regeneration capacities

When the epidermis is cut with a scalpel, the mobilised epidermal stem cells

migrate to the border of the epidermis along the dermo-epidermal junction, divide and differentiate to rebuild the epithelial matrix and close the wound. This is called re-epithelialisation.

Eight days following the cut, re-epithelialisation is much faster in the presence of Phycosaccharide AI than under normal conditions (Fig. 4).

The addition of 5% Phycosaccharide AI optimises the binding of EGF to its receptor EGFR and reinforces the mobilisation and migration of epidermal stem cells. Epidermal regeneration occurs much more rapidly and effectively than under normal conditions.

Revitalising and protecting dermal adult stem cells

Initially, Phycojuvenine 1% significantly revitalises adult stem cells in mature skin (+28% compared to the control, Fig. 5) which recovers a cell vitality equivalent to that of young skin (95% vs 99%). It then has a protective action with respect to the factors of cell ageing such as UVB. From 1%, Phycojuvenine significantly protects the stem cells against ageing: +10% of cell vitality for young skin and +70% for aged skin (Fig. 6).

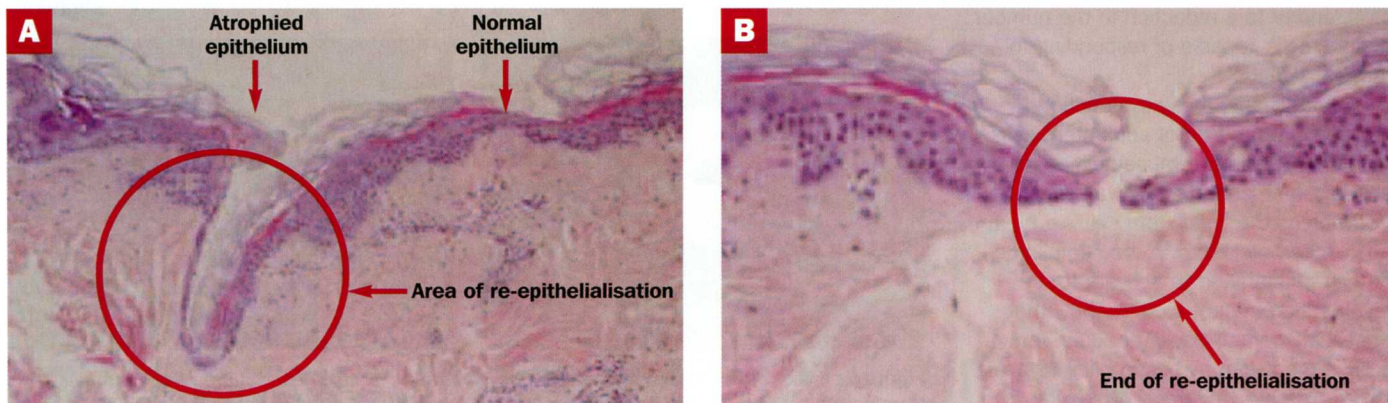


Figure 4: Study of the re-epithelialisation of control skin (4A) and a skin treated with 5% Phycosaccharide AI, 8 days after being cut with a scalpel.

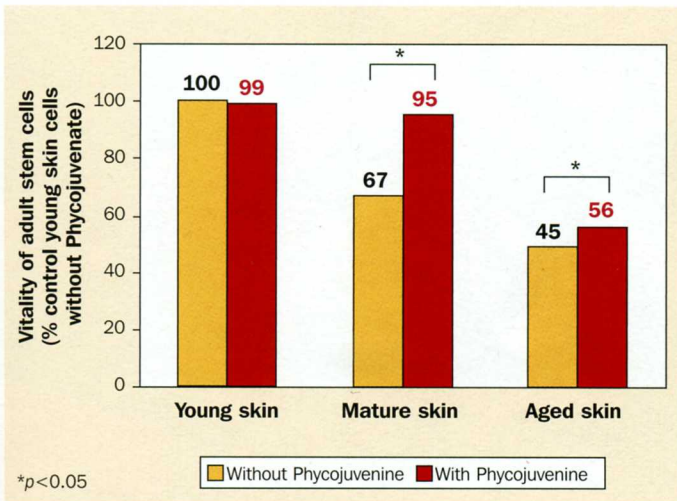


Figure 5: Revitalising effect of 1% Phycojuvenine on adult stem cells in dermis – shown is growth of adult stem cells in 24 hours. Stem cells' vitality was evaluated from the reduction in MTT (tetrazolium salt) by mitochondria.

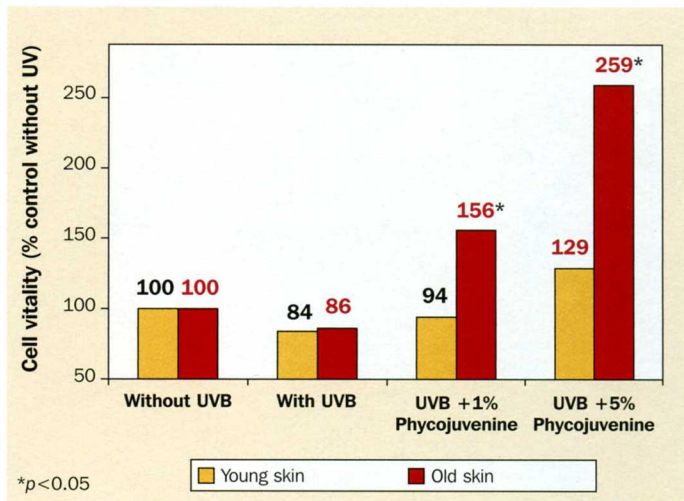


Figure 6: Anti-ageing effect of 1% Phycojuvenine on dermal adult stem cells – the cell ageing process is stimulated by UVB radiation. Adult stem cell cultures were incubated for 24 hours before and after UVB radiation (20 to 30 mJ/cm²). Cell vitality was evaluated from the reduction of MTT (tetrazolium salt) by mitochondria.

This protective effect is more visible on aged skin and compensates for the weakening of the internal defence systems due to skin ageing.

Rejuvenating fibroblasts and collagen synthesis

Fibroblasts grow older and their age may be evaluated by observing their morphology (up to 7 different morphotypes may be counted). Phycojuvenine displaces the average age of fibroblasts subjected to an ageing process from morphotype V to morphotype II to III (Fig. 7). By rejuvenating adult stem cells and then by protecting them from ageing, Phycojuvenine has a rejuvenating effect

on fibroblasts which then produce more collagen: +18% compared to aged fibroblast cultures without Phycojuvenine. Aged fibroblasts then synthesise similar quantities of collagen to young fibroblasts: 137% vs 150% (Fig. 8).

Filling wrinkles

After 28 days of twice-daily use of 1% Phycojuvenine on crow's foot wrinkles, 80% of volunteers observed a significant reduction in the main wrinkle of -6.2% on average and up to -15.6% (Fig. 9).

This reduction in the depth of wrinkles was accompanied by a smoothing effect with a reduction in mean roughness of up to -29.6%.

Phycojuvenine 1% rejuvenates ageing adult stem cells and protects them from ageing. By restoring their vitality and their capacity for division, 1% Phycojuvenine prolongs their efficacy. Dermal fibroblasts are rejuvenated, collagen synthesis of mature skin equals that of young skin, the skin is retightened, and wrinkles are filled and disappear.

'Lasting' and 'controlled' approach

Rather than force cell proliferation, Phycojuvenine and Phycosaccharide A1 open the way to a "lasting" and "controlled" approach in the concept of stem cells, by optimising their mobilisation and

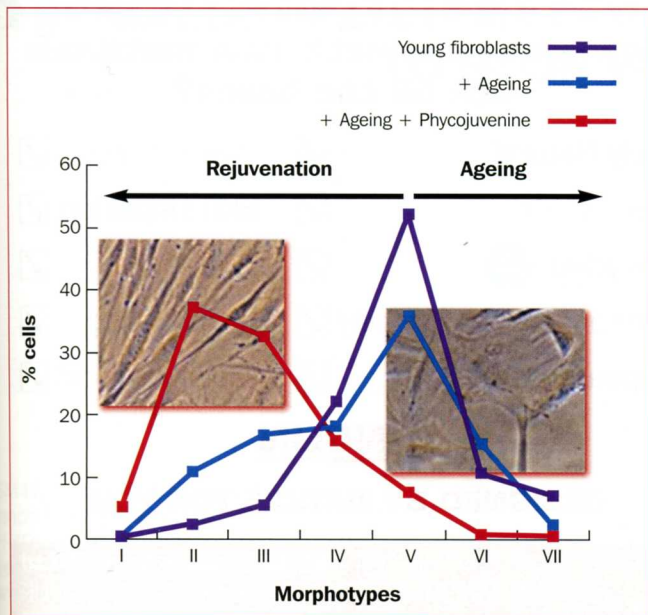


Figure 7: Rejuvenating effect of 5% Phycojuvenine on human fibroblast cultures subjected to an ageing process. Ageing of fibroblasts was induced by UV exposure. The age of fibroblasts is evaluated from their appearance.

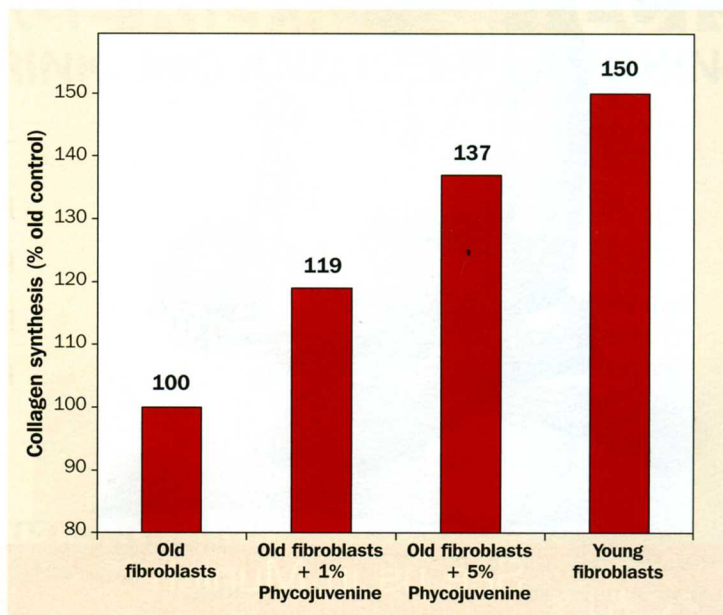


Figure 8: The rejuvenating effect of Phycojuvenine on human fibroblast collagen synthesis was evaluated by following the incorporation of proline which is the main amino-acid in collagen molecules.

