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Vitalisation of dermal stem cells for skin rejuvenation

Stem cells are unspecialised cells with the capacity to self-renew over the whole life period of the organism. When needed, stem cells produce cells that undergo differentiation to specialised tissue cells. Only embryonic stem cells are pluripotent, meaning that they can develop into all cell types of the body, and are thus capable of forming an entire organism. In adult tissues, only multi- or unipotent stem cells are found, indicating that they can differentiate into more than one cell type or only one cell type, respectively. Stem cells are found in virtually all adult tissues where they are responsible for a continuous supply of new cells essential for repair and regeneration. Because adult stem cells are an ethically correct source, research on stem cells of adult tissues has advanced considerably in recent years and new findings are revolutionising the field of regenerative medicine.

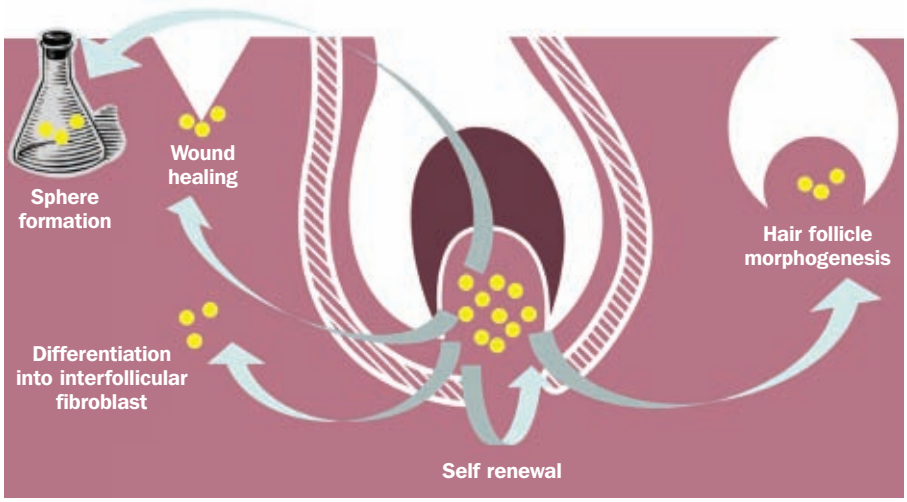

Figure 1: *The dermal papilla as a niche for dermal stem/progenitor cells.*

The rarely explored stem cells of the dermis

In the skin, especially the stem cells found in the epidermis are well characterised. The epidermis is a stratified epithelium that is constantly renewed throughout life. Constant renewal and repair is essential for the maintenance of the normal barrier function, which protects the body from physical and chemical damage, infection and dehydration. Renewal of the epidermis is mediated by stem cells in the basal layer of the epidermis. Protection or stimulation of stem cells in the epidermis has become a hot topic in cosmetics. *In vitro* test systems using epidermal stem cells have been established which allow claims for epidermal stem cell actives. But what about the stem cells in the dermis? Until now, they have not been addressed by cosmetic treatments although they might play a decisive role in skin ageing. Fibroblasts, the prominent cell type in the dermis, are responsible for the continuous production of collagen and elastin. These proteins form the so-called extracellular matrix, a three dimensional structure that confers elasticity and firmness to the skin.

Ageing skin is characterised by an increasing number of senescent fibroblasts. These cells have not only stopped to produce collagen and elastin but even start to break down the existing matrix.[1] The replacement of these senescent cells by new fibroblast cells can only be provided by dermal stem cells. There must be a pool of slowly dividing dermal stem cells. But the number of divisions stem cells of adult tissues can undergo is limited (Hayflick limit), thus, exhaustion of this pool of cells limits the lifespan of the tissue. Treatments that reinforce the functional ability of tissue stem cells, have a real anti-ageing potential.

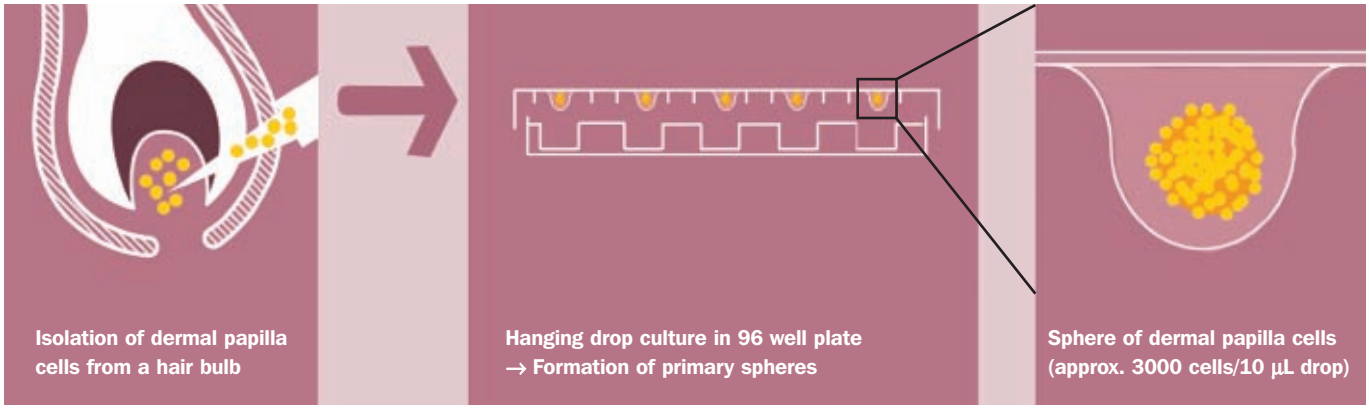

Figure 2: *Sphere formation of isolated dermal papilla cells in hanging drops.*

The dermal papilla is a niche for dermal stem/progenitor cells

Compared to epidermal stem cells, research on stem cells of the dermis is relatively new. In 2001, the first reports about multipotent cells of the dermis appeared.[2] These stem cells were found to be able to differentiate into adipocytes, muscle cells and even neurons. Research was then intensified, because the skin may provide an accessible source of stem cells for transplantation. Experiments about the exact localisation of these multipotent stem cells showed that they are always located near the hair follicle, in the papilla and the perifollicular area, but never appeared in the dermis area between the follicles. But at that time, it was not clear whether these multipotent stem cells could also differentiate into fibroblasts, i.e. acting as dermal stem cells, responsible for maintaining and repairing the dermis. Also, nothing was known about the specific markers of these multipotent stem cells. At the end of 2009, Biernaskie *et al.* showed that dermal papilla cells express the stem cell marker gene Sox2 and have a tendency to grow in colonies in the form of spheres.[3] Sox2 is a transcription factor, shown to be essential to maintain the pluripotent phenotype of stem cells.[4] The Sox2-positive cells were found to self-renew, to induce the formation of hair follicles and to migrate into the inter-follicular dermis where they proliferated and differentiated to fibroblast cells, able to regenerate the extracellular matrix (Fig. 1). For the first time, the dermal papilla was identified as a niche for dermal stem/progenitor cells. The sphere-forming, Sox2-expressing cells are most probably identical to the previously identified multipotent stem cells. The identification of dermal stem cells now opens the door to the next generation of stem cell cosmetics: protection and vitalisation of human dermal stem cells for restoration of skin firmness and wrinkle reduction.

A new assay to measure dermal stem cell activity

Dermal stem cells were isolated from the dermal papilla of excised human hair follicles. These cells were maintained as a monolayer culture for at least 11 passages. At both passage 3 and passage 11 cells transferred into hanging drops formed 3D spheres (Fig. 2),[5] demonstrating that this important characteristic of progenitor cells was retained even after longer-term cultivation. In addition, immunofluorescent labelling of whole mount spheres showed positive staining for the Sox2, a proposed dermal stem cell marker (staining pictures). When cells dissociated from primary spheres were seeded back into classical cell culture dishes used for routine monolayer culture, numerous secondary spheres were spontaneously formed (Fig. 3). This indicates that once cells have formed primary spheres, they seem to retain a memory of the 3D progenitor phenotype, and preferentially re-form spheres where normally monolayer cultures would be expected. In order to evaluate ingredients for a stem cell vitalisation potential, the intensity and uniformity of Sox2-labelling in primary spheres and the number of secondary spheres formed were used as parameters. The assay was used to screen for a dermal stem cell ingredient. An extract of argan-derived stem cells was found to significantly stimulate the vitality of dermal stem cells.

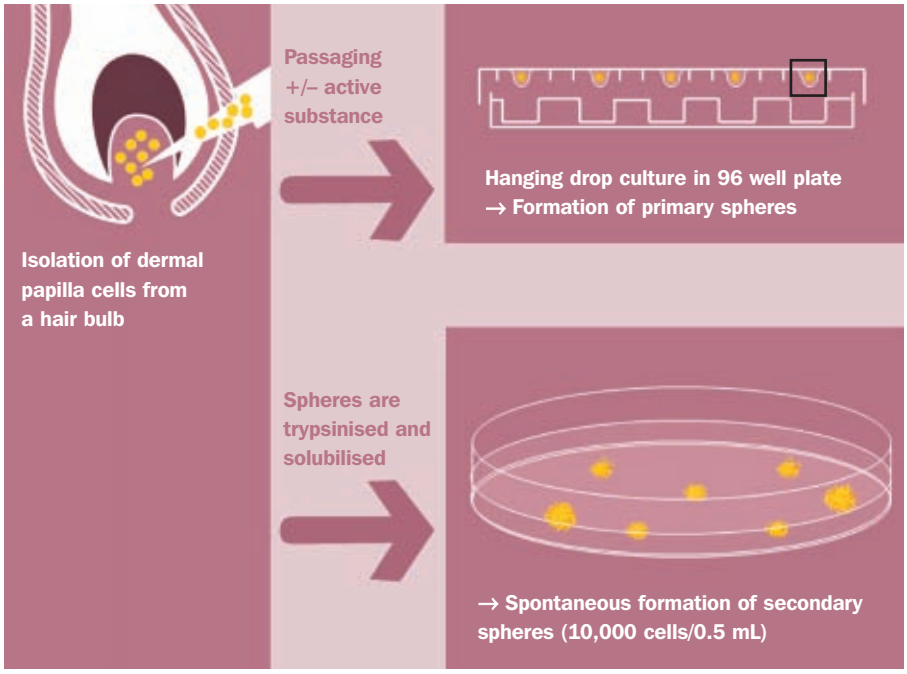

Figure 3: *Formation of secondary spheres as test system for dermal stem cell actives.*

Plant tissue culture technique to produce Argan cell material

The argan tree (*Argania spinosa*) is native in the southwestern regions of Morocco. The tree, perfectly adapted to the arid climate, has an important ecological role as a provider of wood and oil, produced from of its fruit kernels. Argan trees are an endangered species and as such they cannot be used as a raw material for a cosmetic ingredient. Instead, the plant tissue culture technique was used to produce vegetal raw material from argan. The technique is based on the propagation of plant stem cells either to produce a whole plant, only tissue or just single cells in culture to harvest plant metabolites. This practice allows the production of plant material under sterile and standardised conditions independent of season and other environmental restraints. Cultures can be initiated from nearly all plant tissues, beginning with a small tissue sample known as an explant. After wounding, healing at the cut surfaces begins with the formation of a cell mass known as a callus. These cells have dedifferentiated into cells that lack the distinctive features of normal plant cells. Callus cells are stem cells comparable to those in the meristem regions. For high yield production, callus cells can be cultured after homogenisation of the suspended cells in a liquid culture.

Argan shoots were used to start an argan cell culture line. Callus induction and sub-cultivation was carried out according to standard practice. Incorporation of the de-differentiated cells in an appropriate liquid media, homogenisation of the cells in suspension and continuous characterisation of the cell suspension was also carried out according to standard procedures. For up-scaling, 10% of the next larger culture volume of a fully grown cell suspension was used as inoculum. Production of biomass was done in 50 L to 100 L cultures with a special bioreactor-system (Wave-Biotech AG, Tagelswangen, Switzerland). Cultivation was done at 25˚C and an aeration of 0.1 vvm. Biomass production was monitored by analysis of total sugar concentration, conductivity, pH-value and optical density. Production of secondary metabolites was followed by HPLC and UV/VIS analysis. The extract of argan stem cells was obtained after lysis of the plant cells using high pressure homogenisation.

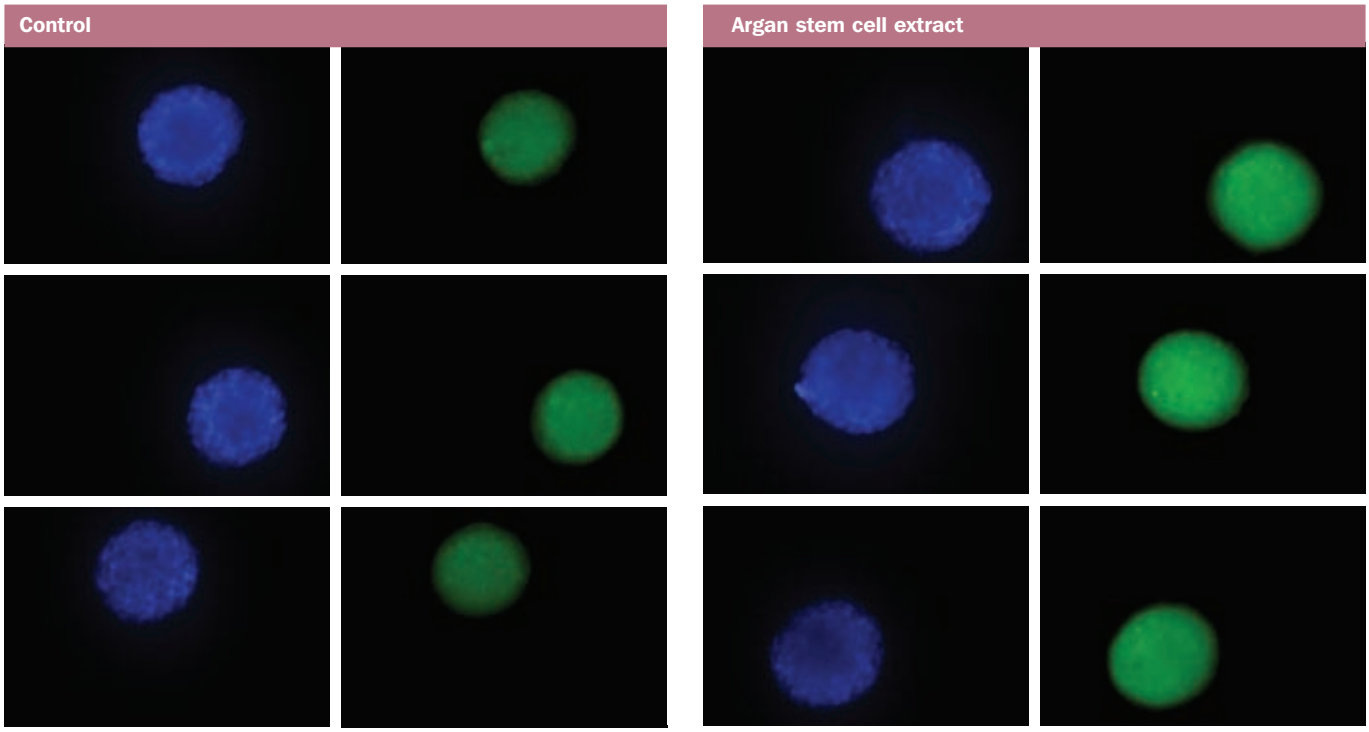

Figure 4: *Immunofluorescence pictures of spheres with DAPI staining for DNA (blue) and Sox2-staining (green).*

Stem cells of the argan tree for vitalisation of human dermal stem cells

Dermal papilla cells, isolated from excised human hair follicles, were cultured over 6 passages in presence of 0.1% of the argan stem cell extract. Cells of passage 9 were used for primary sphere formation in hanging drops. Sixteen days after injection of 3000 cells into 10 µL drops, the primary spheres were prepared for immunohistochemical analysis of expression of the stem cell marker Sox2. The cell nuclei were shown by DAPI staining. Compared to control cultures, the immunofluorescence pictures showed clearly an enhanced expression of Sox2 in spheres formed by dermal papilla cells cultured with the argan stem cell extract (Fig. 4). For the formation of secondary spheres, primary spheres in hanging drops were digested with TrypLE for 30 minutes at 35˚C. The cells were counted and seeded at 10,000 cells per well into 24 well plates. After three weeks, the number of secondary spheres formed was determined. Incubation with the argan stem cell extract was found to stimulate the formation of secondary spheres (Fig. 5). Compared to the control culture, the number was increased by 89%. Overall, the results prove the beneficial effect of the argan stem cell extract on the stem cell characteristics of the dermal papilla cells.

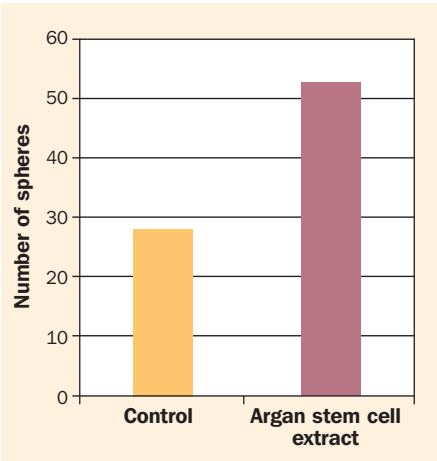

Figure 5: *Number of secondary spheres.*

Conclusion

The perifollicular sheath and the papilla are connective tissue zones around the hair follicle. These areas are well known to contain a pool of multipotent stem cells. In the last decade, it was shown that these stem cells could differentiate into mesenchyme-derived cells such as adipocytes, muscle cells, osteocytes and chondrocytes. Of course, research on these cells was very intense because they are an easily accessible source for stem cell transplantation. Only recently it was shown that the perifollicular sheath and the papilla contain Sox2-positive cells that can reconstitute the dermis and induce hair follicle morphogenesis. These are characteristics of dermal stem/progenitor cells. These findings are not completely surprising because the dermis also represents a mesenchyme-derived tissue. But for the first time a niche for dermal stem/progenitor cells was identified. These cells are very precious for wound healing therapies and represent also an interesting target for cosmetic ingredients. Especially, because over the hair follicle absorption route the dermal papilla zone is accessible for cosmetic active compounds. Protection or even stimulation of the stem cell characteristics of these dermal stem/progenitor cells represents a fundamental anti-ageing approach.

Analysing Sox2-expression and sphere-formation potential, two very typical stem cell characteristics of dermal stem/progenitor cells, an extract of *Argania spinosa* stem cells was identified as a new cosmetic ingredient with beneficial effects on dermal stem cells. PC

References

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