Dermal stem cells are the target of the latest treatments for deep-seated skin regeneration

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Abstract
Fibroblasts 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. Aging skin is characterized by an increasing number of senescent fibroblasts that have stopped producing collagen and elastin. The replacement of these senescent cells by new fibroblasts can only be provided by dermal stem cells. Stem cells are unspecialized cells with the capacity to self-renew over the whole life period of the organism. 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 cells are very precious for wound healing therapies and represent also an interesting target for cosmetic ingredients. In vitro test systems using dermal stem cells were established and allow now claims for dermal stem cell actives. Protection or even stimulation of the stem cell characteristics of these dermal stem/progenitor cells represents a fundamental anti-aging approach as demonstrated in in vitro and clinical studies.

Introduction

The vital cells of the skin

Although the human skin can self-renew by extensive proliferation throughout the life, there is ageing. The cellular mechanisms regulating ageing are still not completely defined, but it is strongly suggested that functional depletion of stem cells results in ageing (Mimeault et al., 2010).
It is well-known that the epidermis sloughs of a layer of cells every day. Therefore, it is clear that the need for new cells is met by stem cell proliferation and by expansion of transit amplifying cells (daughter cells of stem cells) (Winter and Bickenbach, 2009). 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.

Not only cells in the epidermis can age, but also dermal cells, namely fibroblasts. The dermal fibroblasts interact with epidermal (stem) cells and melanocytes regulating their survival and proliferation. Furthermore, the thickness and elasticity of the dermis is controlled by fibroblasts by synthesis of collagen and elastin. These proteins form the so-called extracellular matrix, a three dimensional structure that grants elasticity and firmness to the skin. Aging skin is distinguished by an increasing number of senescent fibroblasts. These cells have not only stopped producing collagen and elastin but even start to break down the existing matrix (Campisi, 1998). The replacement of these senescent fibroblasts by new ones can only be provided by dermal stem cells. Due to the Hyflick limit the number of divisions stem cells of adult tissues can undergo is limited; therefore, exhaustion of this pool of cells limits the lifespan of the tissue. Treatments that strengthen the functional capacity of tissue stem cells have a genuine anti-aging potential.

Dermal precursor cells

Although adult mammalian stem cells were previously thought to differentiate solely into cells of their tissue of origin, a number of recent reports have identified cultured adult stem cells that show a surprisingly diverse differentiation repertoire (Joshi and Enver, 2002).

One population of mesenchymal cells in the skin, known as dermal papilla (DP) cells, is nowadays the focus of intense research in regenerative

medicine and thus of interest for cosmetic treatments (Figure 1). The DP not only regulates hair follicle development and growth, but is also thought to be a reservoir of multi stem cells lineages (Driskell et al., 2011).

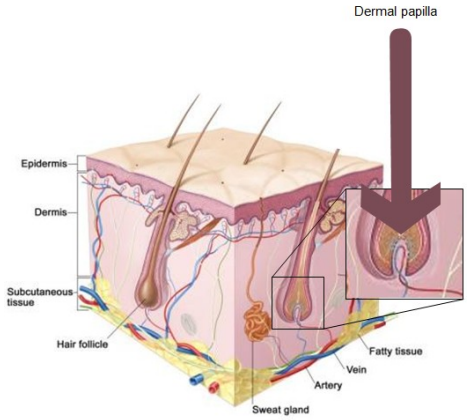

Figure 1: The dermal papillae as niche of dermal precursor cells.

It was shown that dermal papilla cells express the stem cell marker gene Sox2 and are predisposed to grow in colonies in the form of spheres (Biernaskie et al., 2009) (Figure 2).

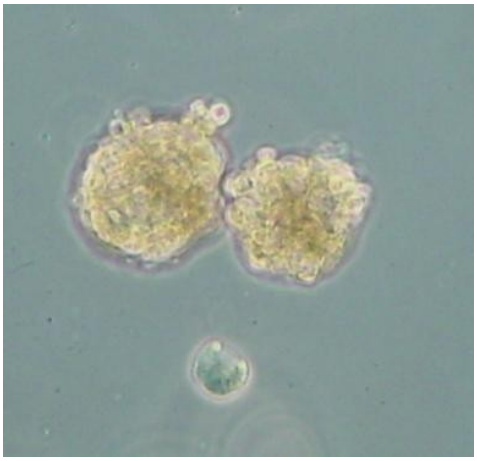

Figure 2: Colonies of dermal papilla cells growing in spheres.

Sox2 is a transcription factor, shown to be essential to preserve the pluripotent phenotype of stem cells. 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.

The identification of dermal precursor cells and the establishment of an in vitro method to culture these cells open the door to the next generation of stem cell cosmetics: fortification and vitalization of human dermal stem cells for re-establishment of skin firmness and wrinkle reduction.

Argan cell culture technique

The argan tree (*Argania spinosa*) belongs to the oldest tree species of the world and grows only in the southwestern part of Morocco. Argan trees are able to resist intense droughts and extremely high temperatures. Nowadays, argan trees are an endangered species and as such they cannot be used as a raw material for a cosmetic ingredient. As an alternative, 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 to produce single cells in culture to harvest plant metabolites. This practice allows the production of plant material under sterile and standardized 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. In this case, argan shoots were used to start an argan cell culture line. Callus induction and sub-cultivation was carried out according to standard practice. The callus cells dedifferentiated into cells that lack the distinctive features of normal plant cells becoming stem cells comparable to those in the meristem regions.

Incorporation of the dedifferentiated cells in an appropriate liquid media, homogenisation of the cells in suspension and continuous cultivation of the cell suspension was also carried out according to standard procedures. Production of biomass was done with a special bioreactor-system (Wave-Biotech AG, Tagelswangen, Switzerland). Biomass production was scrutinized by analysis of total sugar concentration, pH-value, conductivity, and optical density. Production of secondary metabolites was monitored by HPLC and UV/VIS analysis. Finally, the argan stem cells were broken down by high pressure homogenization and used to perform the below mentioned studies.

Study results

Effect of argan stem cell culture extract on dermal progenitor 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 (Figure 3), demonstrating that this important characteristic of progenitor cells was retained even after longer-term cultivation (Higgins et al., 2010). In addition, immunofluorescent labeling of whole mount spheres showed positive staining for the Sox2, a proposed dermal stem cell marker. 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. 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 vitalization potential, the intensity and uniformity of Sox2-labelling in primary spheres and the number of secondary spheres formed were used as parameters. Compared to control cultures, the immunofluorescence pictures showed clearly an enhanced expression of Sox2 in spheres formed by dermal papilla cells cultured in the presence of 0.1% of the argan stem cell extract (Figure 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 3 weeks, the number of secondary spheres formed was determined. Incubation with the argan stem cell extract was found to significantly stimulate the vitality of dermal stem cells (Figure 5).

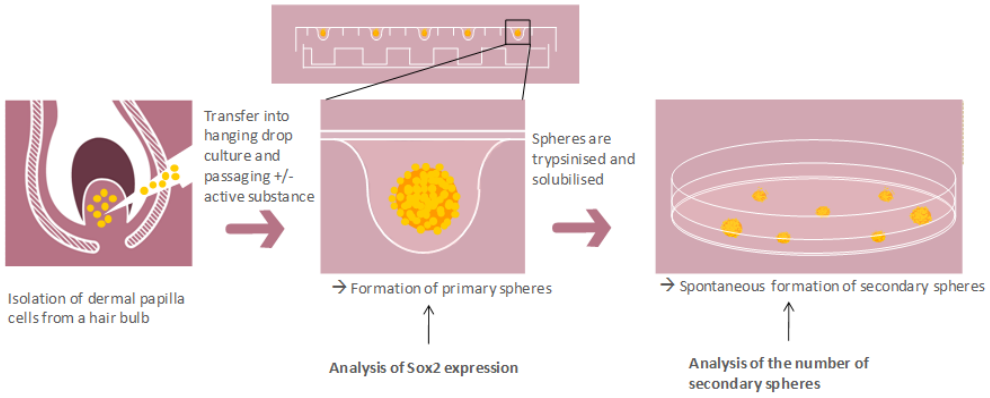

Figure 3: Test system of secondary spheres formation to evaluate dermal stem cell actives.

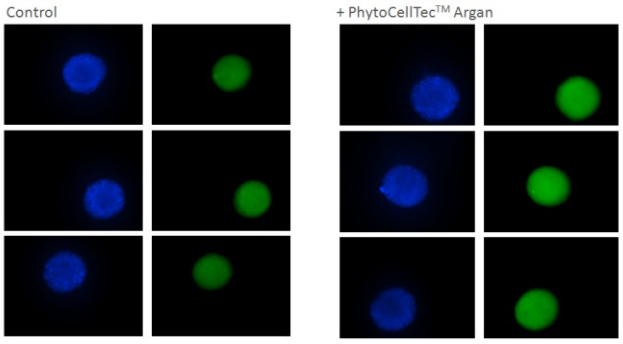

Figure 4: Immunofluorescent light microscopy of full-mounted spheres stained with an antibody against Sox2 (green). Nuclei stained with DAPI (blue).

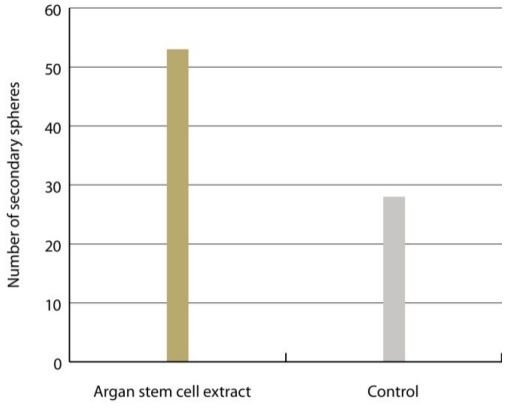

Figure 5: Number of secondary spheres.

Deep-seated regeneration effect on the skin

Evaluation of the tightening effect of PhytoCellTec™ Argan on thigh tissue

A clinical trial was conducted on 22 women aged between 22 and 53 with cellulite grade II-III on thighs. An emulsion with 0.4% PhytoCellTec™ Argan (commercial name of argan stem cell culture extract) was applied twice daily for 56 days on one thigh. The other thigh was treated with the placebo cream. The dermis-hypodermis junction area (DHJA) was determined by ultrasonography by DermScan C. Ultrasonography is a technique for measuring skin density/tightening where pulses of ultrasound waves are emitted into the skin. Reflections which occur from transitions between tissue layers with different acoustic impedance are detected and visualized. In young and tight skin, the dermis-hypodermis area appears as a straight transition, visibly in ultrasonographs as a linear junction between the high echogenic dermis and the low echogenic hypodermis. In the case of cellulite, however, the dermis-hypodermis junction appears as an irregular surface and the dermis-hypodermis junction area is increased.

After 56 days of use, and compared to the placebo product, the emulsion with PhytoCellTec™ Argan induced a decrease in the dermis-hypodermis junction area of 11% (Fig. 6a, b). The skin resulted tighter and smoothness was improved.

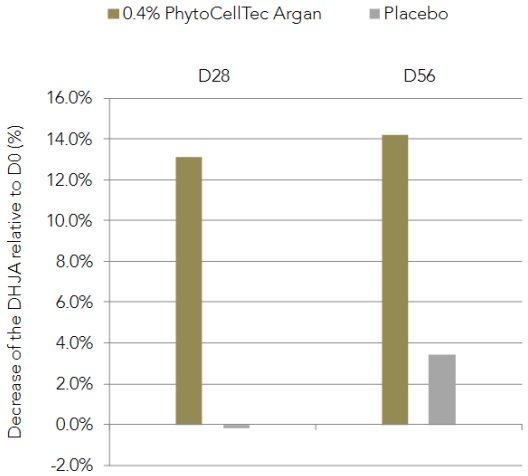

Figure 6a: Dermis-hypodermis junction area after 28 days and 56 days of treatment with PhytoCellTec™ Argan in comparison to the placebo.

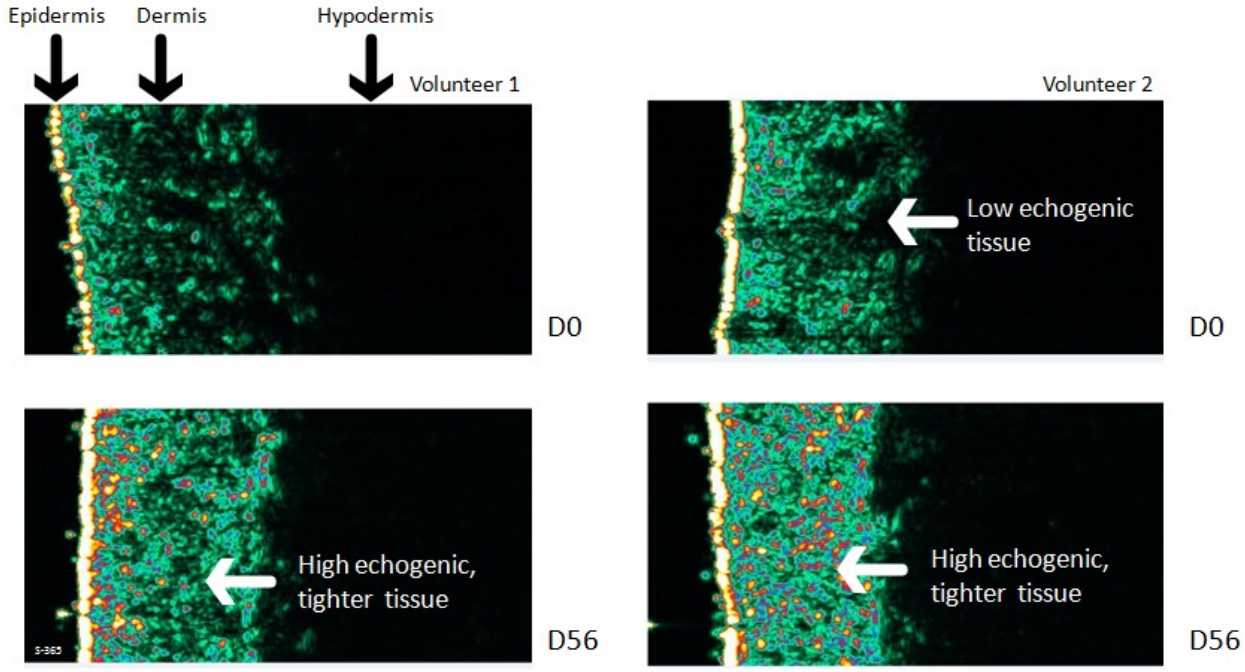

Figure 6b: Ultrasonographic images of women's tights skin at the beginning and at the end of the treatment with argan stem cell culture extract.

Anti-Aging effect of PhytoCellTec™ Argan

A clinical study was carried out over 56 days with 21 women aged from 39 to 61 having sun damaged skin (Phototype I-III). A cosmetic product containing 0.4% PhytoCellTec™ Argan was applied twice per day on the crow's feet area (wrinkle depth) and the inner side of forearms (density) by performing the vehicle-controlled half side comparison. Wrinkle depth was determined by PRIMOS pico and skin density by ultrasonography by DermScan C at days 28 and 56. In the ultrasonography of young skin collagen bundles are homogenous and generate therefore high-echogenic signals in the dermal region. Aged and photo-aged skin shows subepidermal low-echogenic bands (SLEB). Thickness of SLEB increases with age progressively and is greater on sun-exposed skin. Collagen bundles are replaced by a deposit of a greater amount of hydrated proteoglycans, glycosaminoglycans and unbound water.

Results showed that the treatment with argan stem cell extract leaded to an increase in skin density of 12.7% compared to the placebo and after 8 weeks treatment (Figure 7a, b).

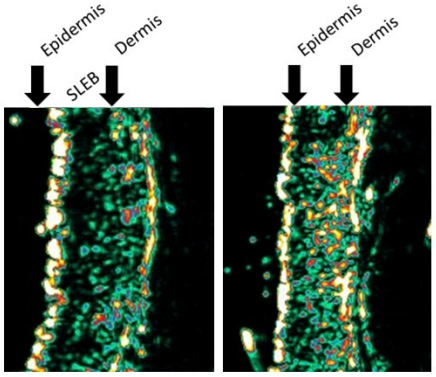

Figure 7a: Density of the upper dermis determined by ultrasonography.

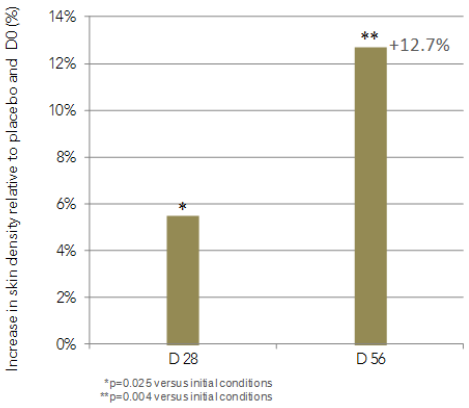

Figure 7b: Increase of skin density compared to the placebo and to initial conditions.

It was also observed that the active successfully reduced the wrinkle depth in crow's feet area by 19% after 28 days of treatment and by 26% after 56 days of treatment (Figure 8).

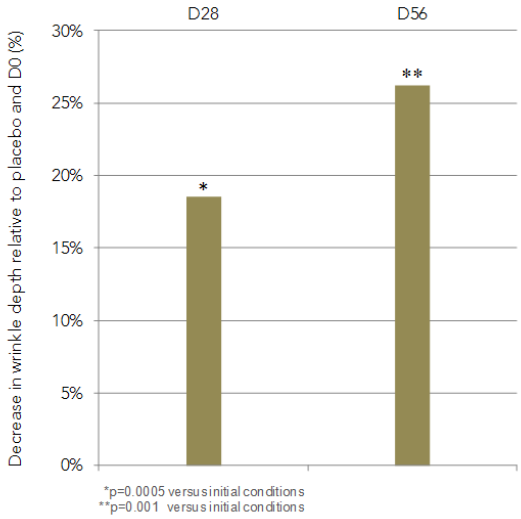

Figure 8: Analysis of wrinkle depth after 28 days and 56 days of treatment relative to the placebo and to initial conditions.

Conclusion

Fibroblast cells are the most prominent cell type in the dermis and responsible for the continuous production of elastin and collagen. These two important structural components build up the extracellular matrix, which gives the skin its properties of elasticity and tensile strength. They have a limited life expectancy and with age, they become less active and decrease in number. As a consequence, senescent fibroblasts are not longer replaced by new ones and the skin loses its elasticity and firmness due to the drop in elastin and collagen production. The ultimate consequence is appearance of wrinkles.

The recently discovered human dermal papilla cells were found to self-renew, to induce formation of hair follicles and to differentiate into dermal cell types like fibroblasts. These cells have been found to specifically express the established stem cell marker Sox2. They also grow in colonies forming three-dimensional spheres which is a dermal stem cells characteristic. Analysis of these parameters showed that the extract of *Argania spinosa* stem cells effectively promotes dermal stem cell activity.

Based on the results obtained by ultrasonographic images after 56 days treatment, argan stem cell extract showed a tightening of the dermal connective tissue compared to initial conditions,

leading to a much smoother dermis-hypodermis junction. Here it was shown a successful method to combat skin dimpling through tightening the connective tissue. PhytoCellTec™ Argan helped the skin to regain its firmness and significantly reduced wrinkle depth in crow's feet area. PhytoCellTec™ Argan is the very first active ingredient able to protect and vitalize human dermal precursor cells at the same time.

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