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Stimuli for skin stem cells for real skin rejuvenation

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ABSTRACT: The fruit of an old apple cultivar were used to establish a plant cell culture line. An extract of these cultured plant cells was tested on keratinocyte progenitor cells. The extract was found to greatly increase the colony-forming efficiency, indicating an enhancement of the stem cell characteristics. The same extract, incorporated at 2 percent into a vehicle cream, was tested in a clinical trial over 4 weeks on 20 subjects. The test cream significantly reduced wrinkle depth in the crow's feet area. The positive effect on skin smoothness could also been shown on digital photos before and after treatment.

INTRODUCTION

When we talk about skin stem cells, we are referring to adult stem cells. They are a self-renewable cell type that exists in most tissues and they form one or several other cell types that develop into differentiated, mature tissue cells. The role of adult stem cells is to maintain and repair tissues. Compared to embryonic stem cells that can differentiate into all cell types of the body, adult stem cells are normally lineage-restricted, meaning that they can only generate the cell types of the tissue in which they are found. But there are many reports of transdifferentiation of adult stem cells into tissue of a different type under the appropriate experimental conditions. Tissue regeneration using adult stem cells is becoming of major interest to medical researchers. Skin stem cells are of special interest because they are easily accessible. Stem cells are also becoming a hot topic in cosmetics. Amatokin, a face care product line of Voss Laboratories, is claimed to stimulate the stem cells in the skin. And Dior's Capture R60/80 XP range are anti-wrinkle products whose mechanism is based on the protection of the life force of stem cells. The skin protects the body from dehydration, injury and infection. The skin consists of an underlying dermis, separated by a basement membrane from the multilayered overlaying epidermis. The dermis is of mesodermal embryonic origin and contains as adult stem cells fibroblastic mesenchymal stem-cell-like cells. These cells have a multi-lineage differentiation potential, being also able to form adipose tissue or bones. The stratified epidermis is of ectodermal origin and composed of keratinocytes that differentiate to a water-impermeable stratum corneum. This article is focused on adult stem cells in the epidermis because they are better characterized than the dermal ones and because ingredients for topical application principally affect the epidermis. The terminally differentiated cells in the epidermis are shed from the skin, necessitating a continuous delivery of newly differentiating cells. The epidermis is completely renewed about every four weeks. Given that the differentiated cells cannot divide anymore, their replacement depends on epidermal stem cells. There is strong evidence that the hair bulge forms a reservoir of epidermal stem cells (Figure 1). From there, stem cells periodically migrate to the matrix of the hair follicle, the sebaceous gland and the basal layer in the interfollicular epidermis (shown with dashed pink lines) to produce progenitors that differentiate into hair cells, gland cells or cells of the upper epidermal layers respectively (shown with dashed green lines) (1).

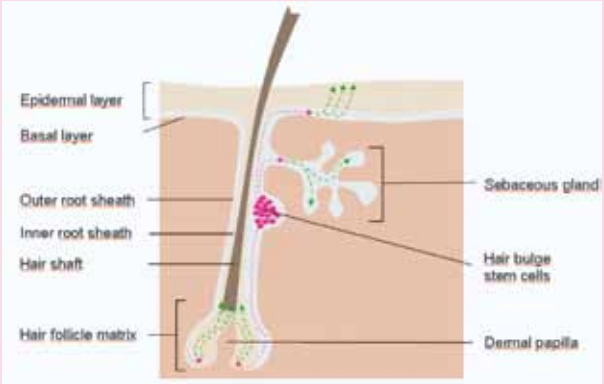


Figure 1. The hair bulge contains multipotent stem cells that contribute to the lineages of the epidermis, the sebaceous gland and the hair follicle.

The basal layer of the epidermis contains two different types of cell populations (Figure 2): (I) the slowly dividing epidermal stem cells and (II) their progeny that are rapidly dividing cells in order to supply new cells to replace those that get lost by desquamation.

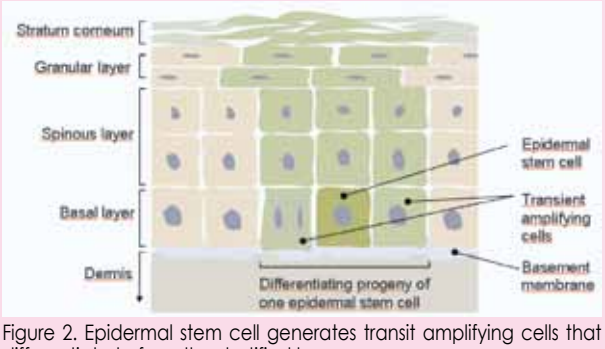


Figure 2. Epidermal stem cell generates transit amplifying cells that differentiate to form the stratified layers.

After a limited number of divisions, they detach from the basement membrane and start the differentiation program leading finally to stratum corneum cells. Newest studies showed that the stem cells might comprise 2-7 percent of the basal layer cells (2). Stem cells manage to keep their undifferentiated status by signals from the niche and by epigenetic control. The niche, composed of the neighbouring, differentiated cells, provides a specific micro-environment. Epigenetic refers to changes in gene expression by mechanisms that are not based on DNA sequence but on modifications of DNA and histones (3). The fact that the self-renewal of epidermal stem cells is slow, is important, because they die after a certain number of divisions and because each division bears the risk of lethal DNA mutations. And loss of stem cells is by far more detrimental for the tissue than when we lose differentiated cells.

Plants also have stem cells. Two populations of stem cells, one comprising the shoot apical meristem and the other the root apical meristem, create the plant body. The maintenance of stem cells in plants is also dependent upon signals from the microenvironment and on similar epigenetic control as in mammalian stem cells (4). The plant tissue culture technique is based on 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 standardized conditions independent of season and other environmental restraints. Plant tissue cultures can be initiated from nearly all plant tissues. The tissue material which is obtained from the plant to culture is called an explant. As a kind of wound reaction, new cells are formed on the cut surfaces of the explant. The cells slowly divide to form a lump of cells which is called 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 homogenization of the suspended cells in a liquid culture.

Apples of an old cultivar, Uttwiler Spätlauber, were used to obtain tissue explants in order to initiate a plant stem cell culture. This apple tree was cultivated because the apples showed excellent storage properties. The Spätlauber variety derives from a seedling that was planted in the middle of the 18th century in Switzerland. A success- full liquid culture in bioreactors of Uttwiler Spätlauber stem cells could be established (5). An extract of these apple stem cells was tested in a series of studies for anti-aging efficacy in skin.

EXPERIMENTAL SECTION

Plant cell cultures

Apples of the Uttwiler Spätlauber variety were chosen as a source of suitable plant material. Callus induction and sub-cultivation was carried out according to standard procedures (Plant cell culture: a practical approach, Ed P. A. Dixon, 1994, Oxford University Press). Incorporation of the dedifferentiated 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 percent of the next larger culture volume of a fully grown cell suspension was used as inoculum. Production of biomass was done in 50 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. For different culturing volumes the respective rocking velocities (20 rocks/min) and rocking angles (8.5 – 9°) were used. 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.

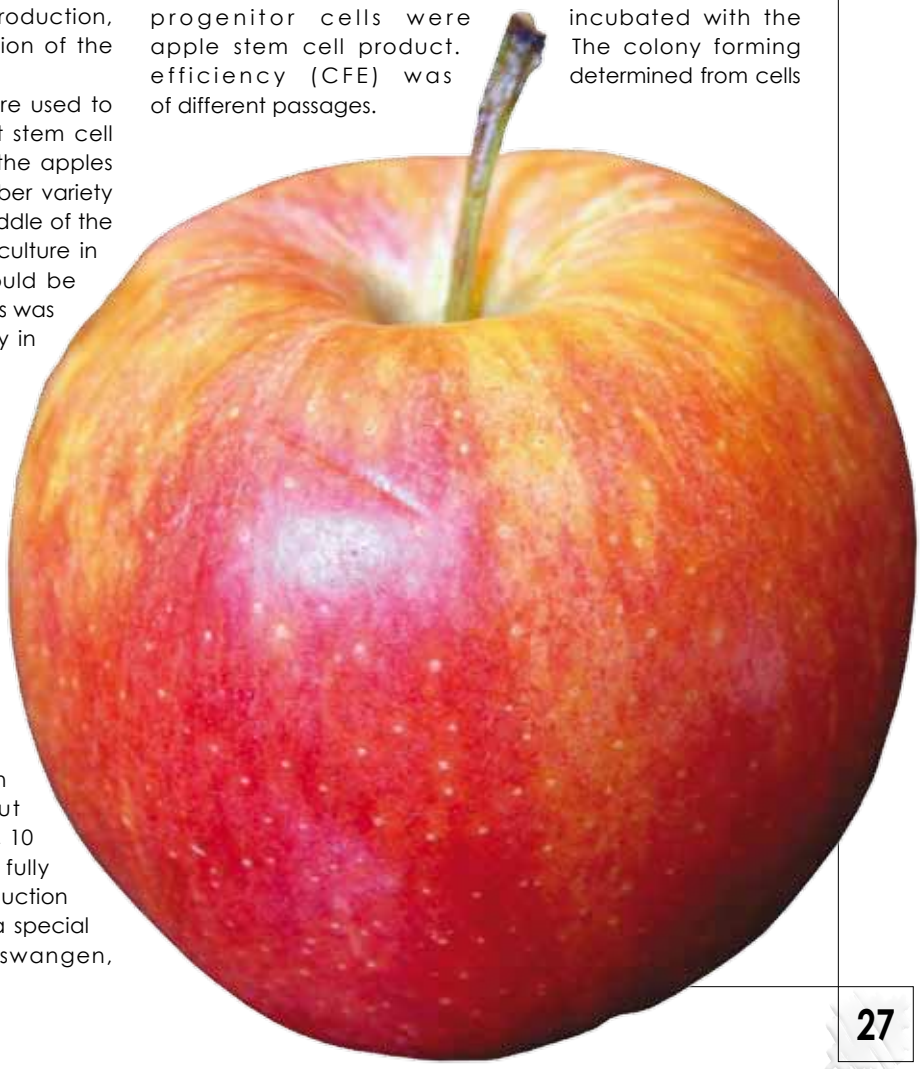
Preparation of a cosmetic ingredient

The extract of Uttwiler Spätlauber stem cells was obtained after lysis of the plant cells using high pressure homogenisation. For preparation of the cosmetic ingredient PhytoCellTec™ Malus Domestica, the extract was incorporated into lecithin liposomes. The composition of PhytoCellTec™ Malus Domestica is the following (INCI): Malus Domestica Fruit Cell Culture Extract, Xanthan Gum, Glycerin, Lecithin, Phenoxyethanol and Aqua.

RESULTS AND DISCUSSION

Effect on epidermal stem cells

The apple stem cell extract was first tested in vitro on epidermal stem cells. A novel Progenitor Cell Targeting technology was used to prepare human epidermal stem cells. The method consists essentially of culturing primary human keratinocytes in a medium specifically designed to mimic the micro-environment of the in vivo stem cell niche. This special, fully defined cell culture medium leads to an enrichment of so called keratinocyte progenitor cells that can be characterized as activated stem cells. Compared to freshly isolated cells, the cell population of passage 4 is characterized by a 10-fold increase of CD34/alpha6 integrin double labelled cells. CD34 and alpha6 integrin are known markers of epidermal stem cells (1). These keratinocyte progenitor cells were incubated with the apple stem cell product. The colony forming efficiency (CFE) was determined from cells of different passages.



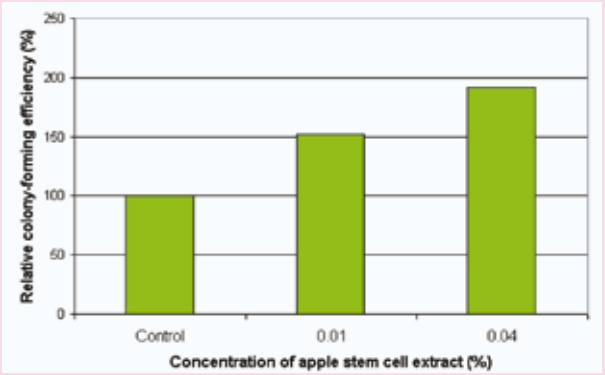


Figure 3. Effect of the apple stem cell extract on colony-forming efficiency of human keratinocyte progenitor cells.

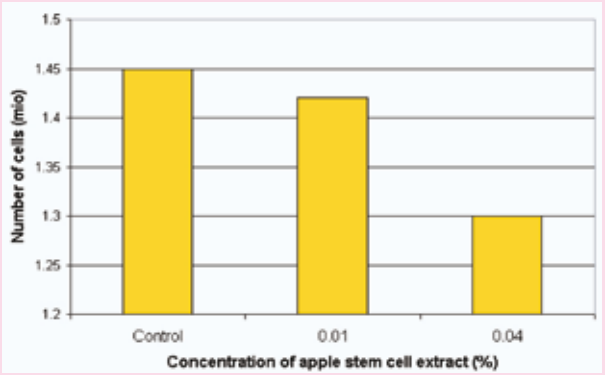


Figure 4. Effect of the apple stem cell extract on proliferation of human keratinocyte progenitor cells.

For analysis of CFE, cells are seeded at low density. The number of colonies formed is a value of the concentration of progenitor/stem cells, because differentiated keratinocytes have lost the capacity to divide and fast dividing transient amplifying keratinocytes do not proliferate enough to form colonies.

Compared to a control culture, the CFE was stimulated by up to 100 percent in the presence of 0.04 percent of the plant stem cell ingredient (Figure 3). This clearly shows that the apple stem cell product improves the maintenance of the stem cell characteristics of epidermal stem cells.

Cell proliferation was reduced in the presence of the apple stem cell product (Figure 4). This again indicates the higher percentage of slowly dividing epidermal stem cells.

Figure 5. Anti-wrinkle effect of a cream with 2 percent PhytoCellTec™ Malus Domestica.

Anti-wrinkle effect on the crow's feet area

The in vivo efficacy of a cosmetic ingredient with the apple stem cell extract was demonstrated in a clinical trial over 4 weeks with 20 women aged between 37 and 64. The test product was a cream with 2 percent PhytoCellTec™ Malus Domestica. The vehicle cream was a normal oil in water emulsion. The test cream was applied twice daily to the crow's feet area. Wrinkle depth was analyzed with the PRIMOS system after 2 and 4 weeks. The PRIMOS is an optical, contact less 3D skin measurement device used for a fast, direct assessment of the skin surface. Digital photos of the crow's feet area were taken at the beginning and the end of the study. The cream with PhytoCellTec™ Malus Domestica was found to significantly reduce wrinkle depth after two and four weeks, by 8 percent and 15 percent respectively (Figure 5). The effect can be nicely demonstrated by the generation of 3D pictures. Such an example is shown in Figure 6. The anti-wrinkle efficacy could also been shown on digital photos.

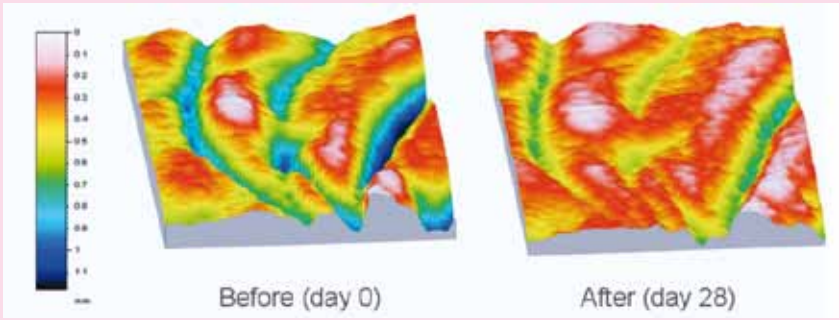


Figure 6. 3D picture analysis of the crow's feet area before and after application of a cream with 2 percent PhytoCellTec™ Malus Domestica.

CONCLUSIONS

Adult stem cells are the source for tissue renewal. Fast regenerating tissues such as the epidermis are especially dependent on them. Adult stem cells are not immortal because they can undergo only a limited number of cell divisions. It is very important that adult stem cells retain their stem cell characteristics. Reduced regeneration potential and finally skin aging is a consequence of the loss of skin stem cell activity. An apple stem cell extract was shown to improve the maintenance of the stem cell characteristics of epidermal stem cells. The anti-aging benefit for skin after topical application could be confirmed in a clinical trial. The exact mechanism is not known but the apple stem cell extract is a promise for real skin rejuvenation.

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