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Formulating Results in Cosmetics R&D

## FRUIT-DERIVED STEM CELLS FOR SKIN CARE

Growth Factors to Boost Antiaging  
Assessing Propanediol for Skin Effects  
Nanoparticles and Skin Penetration

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# Stimulating Epidermal Regeneration with Plant-derived Stem Cells

Daniel Schmid, PhD, and Fred Zülfi, PhD

Mibelle Biochemistry, Buchs, Switzerland

**KEY WORDS:** *epidermal and plant stem cells, colony forming efficiency, three-dimensional epidermis, UV protection*

**ABSTRACT:** *Here, the authors describe mechanisms of stem cells and their potential for antiaging benefits. Cultures established from apple and grape varieties using a specialized culture technology revealed that aside from stimulating colony forming efficiency (CFE) and organogenic potential, the extracts improved the maintenance of epidermal stem cells. In addition, the grape extract provided UV protection.*

Stem cells possess three key properties: they are unspecialized, they can renew themselves over time and they can develop into cells with specific functions. Stem cells are broadly classified into two types—embryonic and adult stem cells. Embryonic stem cells are pluripotent, meaning they can develop into all cell types in the body and are therefore capable of forming an entire organism. In contrast, adult stem cells generally are multipotent and have the ability to develop into the different cell types within the tissue in which they are found, a process also known as differentiation. Adult stem cells are found in virtually all tissues. Through asymmetrical division, adult stem cells maintain a pool of their own cell type to provide continual repair and regeneration benefits for an organism throughout its life span. Without a pool of effective, proliferating adult stem cells, the continual loss of fully differentiated cells cannot be replenished and the tissue soon loses the ability to function.

## Stem Cell Research

In recent years, stem cell research has advanced considerably and new findings have revolutionized the field

of regenerative medicine. Traditionally, embryonic stem cells were believed to be the only source of pluripotent cells. However, recent discoveries have shown that it also is possible to restore adult stem cells or differentiated cells to a pluripotent state. Early methods to restore pluripotency or dedifferentiation required substantial genetic modification of the cell, typically using a virus to insert specific genes. The latest experimental results have shown that restoring pluripotency is also

possible without altering the underlying DNA sequence. These methods typically use a defined group of small molecules to establish the required gene expression pattern through an intricate web of normal epigenetic control mechanisms that do not alter the DNA.<sup>1-3</sup>

## Stem Cells and Aging

Declining regenerative potential at the tissue level is a major contributor to the aging process. Since regeneration depends upon adult stem cells to supply the new cells required for tissue repair and replacement, any decline in stem cell activity will directly facilitate the aging process. Research has shown that mechanisms specifically involved in protecting normal cell function, i.e., telomerase, P53 and others, are degraded over time by exposure to UV radiation, chemical radicals, etc. These weakened protective mechanisms lead to degraded genomic integrity, which in parallel with disrupted epigenetic control, leads to altered gene expression and stem cell function. However, research suggesting that stem cells could

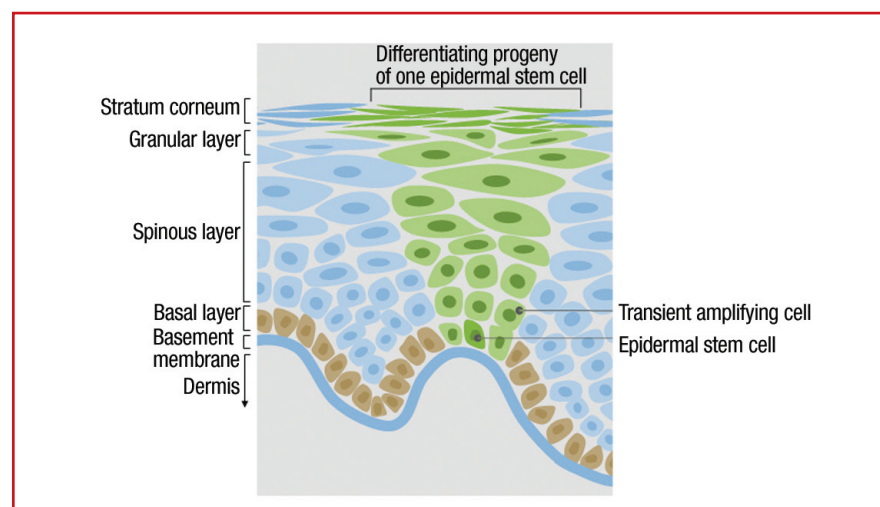


Figure 1. Epidermal stem cells produce more rapidly dividing transient amplifying cells



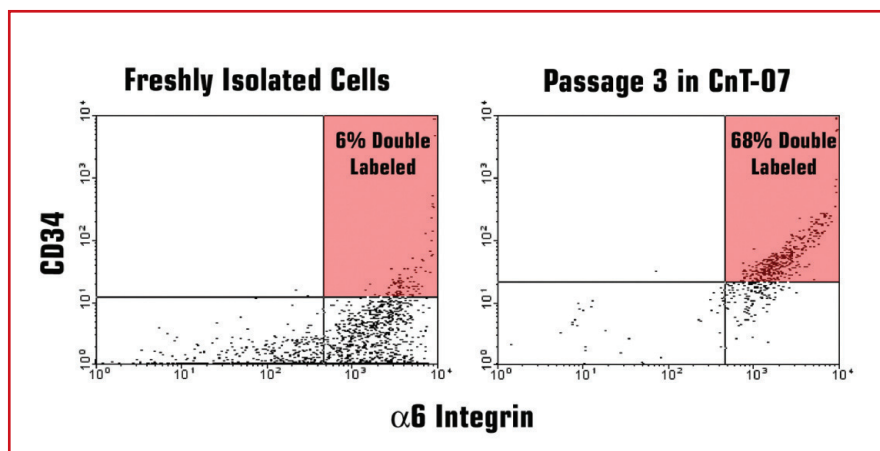


Figure 2. Cells expressing CD34 and alpha6-integrin after 3 passages

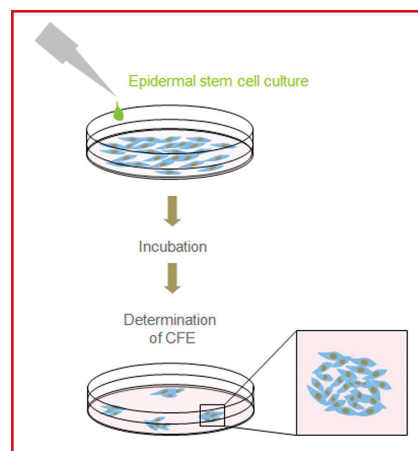


Figure 3. Analysis of CFE

### Formula 1. Grape stem cell extract sun cream

Water ( <i>aqua</i> )	qs to 100% w/w
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) propylparaben (and) isobutylparaben	1.00
Magnesium aluminum silicate	1.00
Galactarabinan	0.20
Methylene bis-benzotriazolyl tetramethylbutylphenol	3.00
Diethylhexyl carbonate	5.00
Ethylhexyl methoxycinnamate	4.00
C <sub>12-15</sub> alkyl benzoate	2.00
Titanium dioxide	0.50
Isostearic acid	0.30
Glyceryl stearate citrate	1.50
Cetearyl alcohol	1.00
Glyceryl stearate	0.50
Myristyl myristate	0.30
Ethylhexyl triazone	0.30
Xanthan gum	0.50
Fragrance ( <i>parfum</i> )	0.25
<i>Vitis vinifera</i> (grape) fruit cell extract (and) isomalt (and) lecithin (and) sodium benzoate (and) water ( <i>aqua</i> ) (PhytoCellTec Solar Vitis, Mibelle)	0.40

be reprogrammed by adding specific factors to their environment may mean that treatments affecting epigenetic controls could stimulate the function of adult stem cells, thus imparting antiaging benefits.

## Epidermal Stem Cells

As is well-known, the epidermis is a stratified epithelium that is constantly renewed throughout life. This constant renewal and repair is essential for maintenance of normal barrier function, which protects the body from physical and chemical damage, infection

and dehydration. The turnover time of the epidermis is approximately 40–56 days.<sup>4</sup>

Turnover of the epidermis is mediated by undifferentiated stem and progenitor cells, which reside in the basal layer of the epidermis (see **Figure 1**). These cells represent approximately 2–7% of the total cells in the epidermis. Undifferentiated cells in the epidermis can be identified *in vivo* via label-retention studies enabling detection of slow-cycling cells, and by the expression of specific marker proteins such as alpha6-integrin and CD34.<sup>5</sup>

Following asymmetrical division, epidermal stem cells produce more rapidly dividing transient amplifying cells, which after a limited number of divisions, enter terminal differentiation. As the cells differentiate, they migrate up through the epidermis, finally forming the uppermost stratum corneum (SC). At this point, the cells have ceased proliferation, lost their nuclei and serve only as a physical barrier. With age, the turnover of the epidermis is reduced, making it thinner, more fragile, and more likely to suffer from impaired wound healing. It has been found that epidermal cells isolated from older donors have a lower stem cell function than epidermal cells originating from younger donors.<sup>6</sup>

## Measuring Stem Cell Activity

It is difficult to demonstrate that the stimulation of epidermal stem cell activity results in skin rejuvenation due to the low prevalence of stem cells in the epidermis, which makes it difficult to evaluate the responses of these specific cells. However, a new technology<sup>a</sup> has made it possible to establish enriched cultures of progenitor/stem cells taken from a skin sample. This technique uses specialized culture media to mimic the environment of the stem cell niche. Fluorescence-activated cell sorting (FACS) analysis has demonstrated that this technology selects and enriches the undifferentiated cells found in primary

<sup>a</sup>Progenitor Cell Targeted culture media is developed by CELLnTEC Advanced Cell Systems, Bern, Switzerland.

keratinocyte cultures. For example, the percentage of cells expressing the markers CD34 and alpha6-integrin increased from 6% to 68% during the first three passages of culture, as shown in **Figure 2**.

Using the described enriched cultures, researchers evaluated the ability of two fruit stem cell-derived extracts<sup>b,c</sup> to maintain epidermal stem cell potential. The enriched cell population was cultured for different time periods in a medium containing one of the ingredients at various concentrations. The epidermal stem cell potential was then evaluated by assessing colony forming efficiency (CFE) and/or organogenic potential, i.e., the ability to form a multilayered epidermis.

For analysis of CFE, cells are seeded at low density and allowed to form colonies, as shown in **Figure 3**. Colonies

are then counted and the percentage of seeded cells that formed colonies is calculated. This percentage gives a direct indication of the number of cells with stem cell potential, since only the undifferentiated cell types retain the ability to form colonies. To determine the organogenic potential, cells are seeded onto a permeable substrate and cultured at the air-liquid interface to encourage the establishment of a three-dimensional, multilayered epidermal structure.

### Plant-derived Test Actives

Two active ingredients were developed using the described culture technology and their effects on epidermal stem cells were tested. The first ingredient was derived from the Uttwiler Spätlauber apple<sup>a</sup>, a variety known for its longevity since it can be stored for long periods of time without shriveling; it was therefore hypothesized to have long-lasting stem cells. The second ingredient, derived from the Gamay grape variety<sup>b</sup>, is known for its high antioxidant content and was therefore examined for its potential UV protective capability.

Unlike humans, adult plants contain totipotent stem cells that have the potential to differentiate into all other tissues of the plant or even regenerate the entire plant. Therefore, in order to harvest plant metabolites, plant stem cells can be processed via the same tissue culture technology described to produce single cells, tissues or whole plants. This method allows for the production of plant materials under sterile and standardized conditions, independent of season and other environmental restraints.

**Declining regenerative potential at the tissue level is a major contributor to the aging process.**

Cultures can be initiated from nearly all plant tissues, beginning with a small tissue sample known as an explant.<sup>®</sup> After a plant is wounded, healing at the

<sup>b</sup>PhytoCellTec Malus Domestica (INCI: Malus Domestica Fruit Cell Culture Extract) is a product of Mibelle Biochemistry, Buchs, Switzerland.

<sup>c</sup>PhytoCellTec Solar Vitis (INCI: Vitis Vinifera Fruit Cell Extract) is a product of Mibelle Biochemistry, Buchs, Switzerland.



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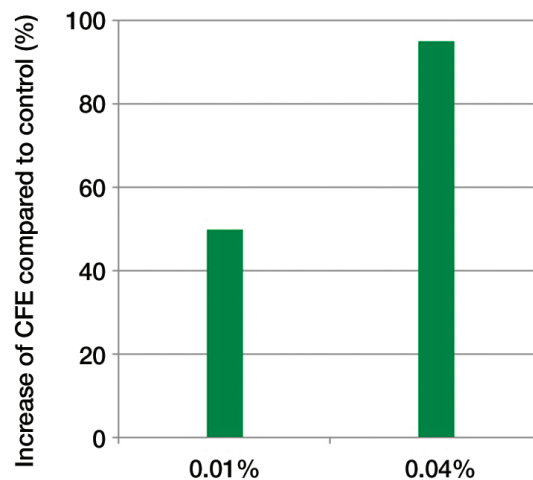
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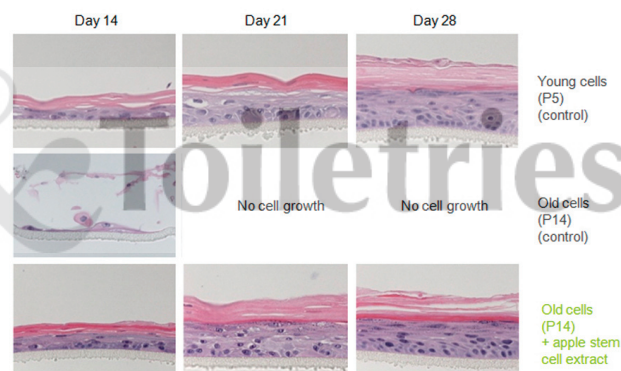
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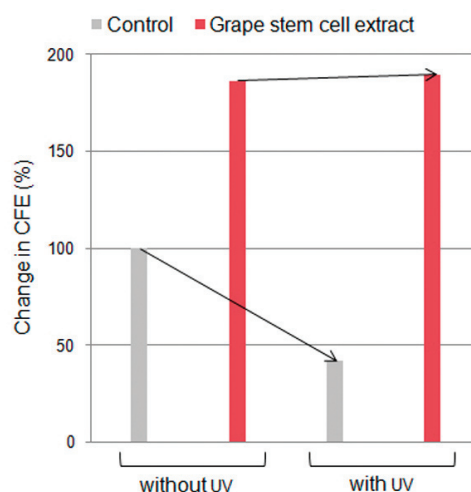
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**Figure 4. Apple stem cell extract stimulates the CFE of epidermal stem cells**



**Figure 5. Apple stem cell extract maintains the capacity to form organogenic potential, i.e., a pluristratified epidermis**



**Figure 6. Grape stem cell extract stimulates the CFE of epidermal stem cells and protects against UV-induced loss of function**

surfaces of the cut begins with the formation of a cell mass known as a callus. Callus cells are a dedifferentiated cell types that lack the distinctive features of normal plant cells, and these cells share many features of meristematic cells; i.e., the plant equivalent of animal stem cells. In high yield liquid culture, callus cells can be cultured either individually or as clusters.

## Effects on Stem Cells

**Apple stem cell culture:** Treatment of

the enriched epidermal stem cells with the apple stem cell culture<sup>b</sup> was found to stimulate both CFE and organogenic potential. Compared with a control culture of epidermal stem cells cultured in a medium without the extract, the CFE was increased by up to 100% in the presence of 0.04% apple stem cell extract, as shown in **Figure 4**.

In addition, treatment with the apple stem cell extract extended the age at which epidermal cells were still able to form a three-dimensional epidermal

structure in vitro (i.e., the organogenic potential). This age was measured by passage. At each passage, the group of cells grown in culture-containing medium are transferred into a flask with fresh medium, where they begin to proliferate again. With every passage, cells get older; so while passage 5 cells are young, at passage 15, cells begin to senescence.

Aged cells (passage 14) were unable to form the three-dimensional epidermal structure. However, aged cells that had been maintained in the presence of the apple stem cell extract exhibited no loss of function and were able to establish a normal three-dimensional epidermal structure even at this advanced age. This continued functioning of the epidermal stem cells shows that the apple stem cell extract improved the maintenance of the epidermal stem cells. As a comparison, a young culture of epidermal stem cells (passage 5) was found to generate a three-dimensional epidermis both in the presence and absence of the apple stem cell extract, as shown in **Figure 5**.

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**Apple stem cell extract extended the ability of epidermal cells in vitro to form 3-D epidermal structures.**

**Grape stem cell extract:** The treatment of epidermal stem cells with a grape stem cell extract<sup>c</sup> was found to increase CFE by 86%, as shown in **Figure 6**. In addition, this extract was found to provide protection against UV exposure. A UV dose that reduced CFE in untreated control cells by 50% was found neutralized by the grape stem cell extract (data not shown). The grape stem cell extract also was formulated into a sun cream (see **Formula 1**) that was found to reduce UV-induced erythema in a placebo-controlled clinical study (data not shown).

## Conclusion

This study is perhaps the first time plant stem cells were tested on isolated stem cells of the human epidermis. The stem cell extracts of apple and grape were found to protect and enhance the



stem cell potential of epidermal stem cells. Formulated into skin care products, these ingredients could benefit the epidermis of mature consumers by improving its skin regeneration abilities to normalize the barrier function and thus prevent or slow the signs of premature aging. In addition, whereas the apple stem cell extract provided general rejuvenation efficacy, the grape stem cell extract imparted skin protection abilities against UV damage.

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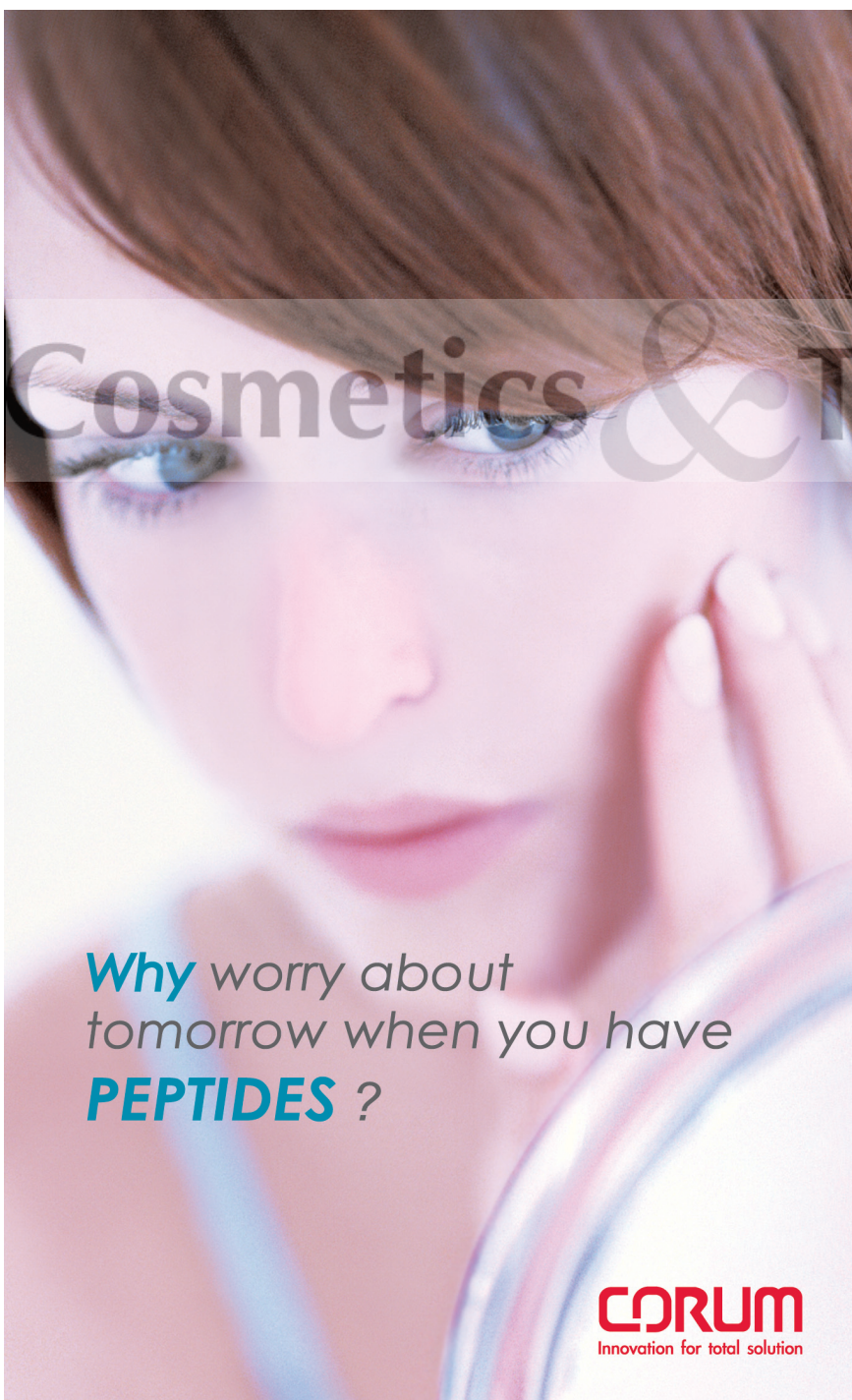
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Send e-mail to [daniel.schmid@mibelle.ch](mailto:daniel.schmid@mibelle.ch).

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