An apple a day

An apple stem cell extract has already been shown to maintain the youth of human skin stem cells. Now Daniel Schmid presents new studies performed with isolated human epidermal stem cells demonstrating how the extract improves the maintenance of epidermal stem cells

Stem cells are unprogrammed cells with two defining properties: the capacity to replenish themselves through self-renewal and the ability to differentiate into cells with specific functions. Stem cells play a central role in the development of a new organism and in the repair and regeneration of tissues. A new organism is formed by embryonic stem cells. They are pluripotent, which indicates that they can differentiate into all cell types of the body. Tissue repair and regeneration is the job of adult stem cells. They are more restricted and are normally only able to differentiate into the different cell types of the tissue in which they are found.

Self-renewal is the ability of stem cells to divide and produce more stem cells. During early development the cell division is symmetrical, which means that two identical stem cells are formed. Later in development the cell division becomes asymmetrical, with one daughter cell being an identical stem cell and the other one representing an undifferentiated progenitor cell (figure 1). The progenitor cells in turn produce differentiated cells through subsequent rounds of proliferation. Asymmetrical division is typical for adult stem cells.

STEM CELLS IN AGEING

The decline in the regenerative potential of tissues is the principal cause of ageing. Since the regenerative process of a living organism is determined by the ability of its stem cells to replace damaged tissue or worn out cells, ageing is directly dependent on the functional ability of tissue stem cells. Normally the number of divisions a cell can undergo is limited, a phenomenon known as the Hayflick limit. The biological clock is the length of telomeres that are repeated DNA sequences at the end of the chromosomes. With each cell division, telomeres are shortened. When telomeres reach a critical length, cell proliferation is stopped and the cell enters senescence.

Thus ageing could be the result of stem cell exhaustion because of the Hayflick limit. But adult stem cells are also subject to premature senescence because of DNA or protein damage. Treatments that stimulate the functional ability of tissue stem cells have a real anti-ageing potential.

STEM CELLS IN THE SKIN

The epidermis contains three different populations of stem cells: hair follicle stem cells, sebaceous gland stem cells and the interfollicular stem cells. The dermis contains mesenchymal stem cells. This article concentrates on interfollicular stem cells that for simplification are just called epidermal stem cells. The epidermis is a stratified epithelium that is constantly renewed throughout life. The constant renewal and repair is important to maintain the barrier function of the epidermis. The barrier protects the body from physical and chemical damage, infection and dehydration. The turnover time of the epidermis is at about 40-56 days (Kostner 2009).

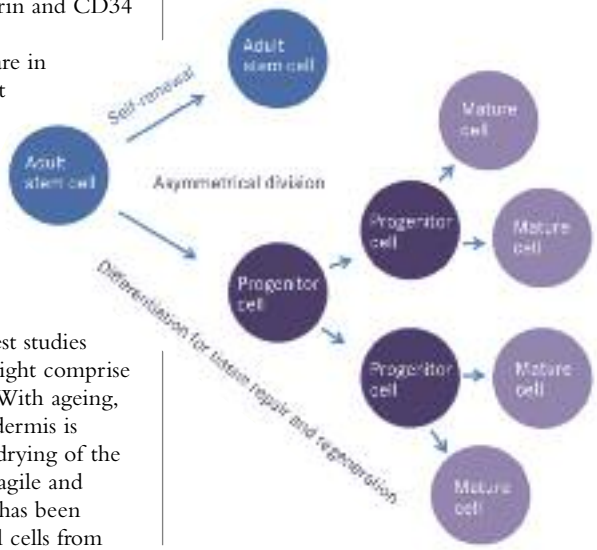
This constant renewal is mediated by epidermal stem cells. They have been identified on the basis of long-term self-renewal ability in culture and because of the expression of specific surface marker proteins such as alpha6 integrin and CD34 (Yan and Owens 2008).

The epidermal stem cells are in vivo slowly dividing cells that are dispersed in the basal layer (figure 2). They produce rapidly dividing (transient amplifying) cells. After a limited number of divisions, they start the differentiation programme leading finally to stratum corneum cells. Newest studies showed that the stem cells might comprise 2-7% of the basal layer cells. With ageing, the turnover time of the epidermis is reduced. This change causes drying of the epidermis, making it more fragile and impairing wound healing. It has been found that isolated epidermal cells from older donors have a lower stem cell function than epidermal cells originating from younger donors (Barrandon and Green 1987).

APPLE STEM CELLS

Apples with very good storage properties stay fresh over months. These apples must have especially long living tissue stem cells. Could we profit from these stem cells? What would be the effect of an extract of

FIGURE 1
SELF RENEWAL AND DIFFERENTIATION OF ADULT STEM CELLS



such long living stem cells on the skin?

Commercially available apples are not suitable for long storage however. They are selected for intensive cultivation and for a pleasant sweet flavour. In former times, good storage properties were an important factor for cultivar selection. Some of these old cultivars survived as isolated trees in areas with less intensive agriculture.

The Uttwiler Spätlauber is an apple tree that was cultivated especially because of the good storage properties of the fruit. The Spätlauber variety derives from a seedling that was planted in the mid 18th century. There are still some Uttwiler Spätlauber trees left in certain areas in Switzerland and the apples of one of these trees were used to obtain tissue explants in order to initiate a plant cell culture. Adult plants contain totipotent tissue stem cells with the potential to regenerate a whole plant.

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 standardised conditions independent of the season and other environmental restraints. Uttwiler Spätlauber stem cells were successfully cultured in a liquid medium in a disposable bioreactor system (Wave-Biotech AG, Switzerland) (figure 3). An extract of these apple stem cells was used as the principal active for the commercial product PhytoCellTec Malus Domestica.

Effect of apple stem cell extract on epidermal stem cells

A novel Progenitor Cell Targeting technology was used to isolate human epidermal stem cells. Primary human keratinocytes were cultured in a special, fully defined cell culture medium that made an enrichment of epidermal stem cells possible. The high concentration of

FIGURE 2 NEW CELLS PROVIDED BY EPIDERMAL STEM CELLS AT THE BASAL LAYER

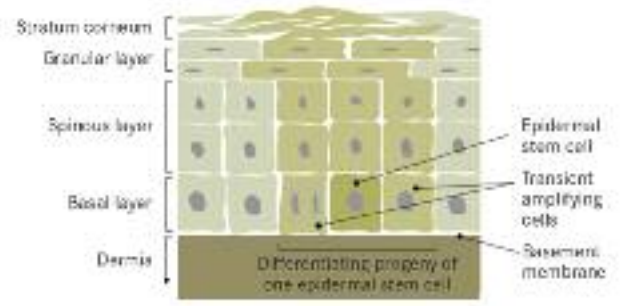
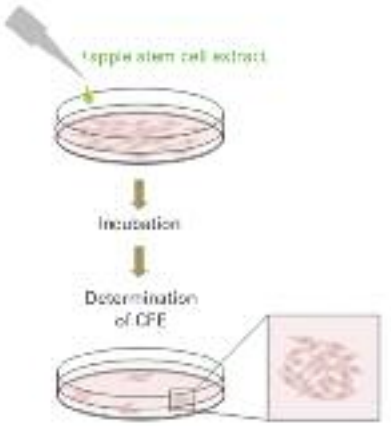


FIGURE 3 BIOREACTOR SYSTEM WITH DISPOSABLE BAGS

FIGURE 4
DETERMINATION OF COLONY FORMING EFFICIENCY



stem cells was confirmed by analysis of the surface marker proteins alpha6 integrin and CD34. This culture was used to test the effect of an extract of the apple stem cells on maintaining the epidermal stem cell potential.

The epidermal stem cells were cultured for different time periods in a medium containing the extract at various concentrations. Two different methods were used to analyse the epidermal stem cell potential: determination of colony forming efficiency (CFE) and analysis of the potential to form a pluristratified epidermis (organogenic potential). For analysis of CFE, cells are seeded at low density (figure 4).

The number of colonies formed is a measure of the concentration of 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% when epidermal stem cells were cultured in the presence of 0.04% of the apple stem cell extract (figure 5).

The longer the epidermal stem cells were cultured in the presence of the apple stem cell extract, the more pronounced was the stimulatory effect on CFE compared to a control culture (figure 6). The organogenic potential is another indicator of the number and activity of stem cells. Cells of a culture were seeded onto a dermal substrate and analysed for their potential to build up a three dimensional epidermal structure (3D epidermis). The formation of the epidermis was followed by microscopic analysis of cross sectional areas at days 14, 21 and 28 (figure 7).

FIGURE 5
APPLE STEM CELL EXTRACT STIMULATES COLONY FORMING EFFICIENCY OF EPIDERMAL STEM CELLS

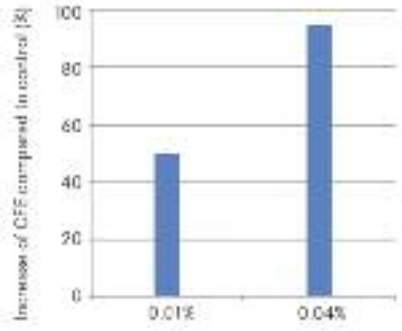
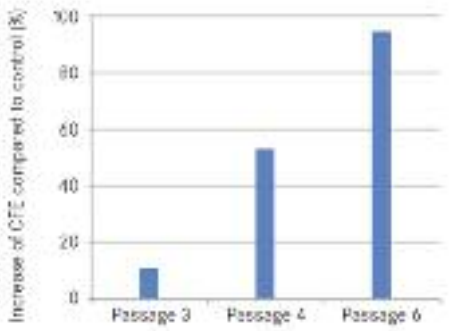


FIGURE 6
STIMULATION OF COLONY FORMING EFFICACY OVER SEVERAL PASSAGES



A young culture of epidermal stem cells (P5) is able to form a perfect 3D epidermis. But cells that are cultured over several weeks (P14) have normally lost their stem cell potential and are no longer able to form a pluristratified epidermis (figure 7, old cells, control). Surprisingly, cells that are cultured over the same time in presence of the apple stem cell extract were perfectly able to form a 3D epidermis (figure 7, old cells + apple stem cell extract). This clearly shows that the apple stem cell extract improves the maintenance of the stem cell characteristics of epidermal stem cells. **cb**

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FIGURE 7
APPLE STEM CELL EXTRACT PRESERVES CAPACITY TO FORM A 3D EPIDERMIS