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REVIEW

PLANT STEM CELLS AS INNOVATION IN COSMETICS

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Abstract: The stem cells thanks to their ability of unlimited division number or transformation into different cell types creating organs, are responsible for regeneration processes. Depending on the organism in which the stem cells exists, they divide to the plant or animal ones. The later group includes the stem cells existing in both embryo's and adult human's organs. It includes, among others, epidermal stem cells, located in the hair follicle relieves and also in its basal layers, and responsible for permanent regeneration of the epidermis. Temporary science looks for method suitable for stimulation of the epidermis stem cells, amongst the other by delivery of e.g., growth factors for proliferation that decrease with the age. One of the methods is the use of the plant cell culture technology, including a number of methods that should ensure growth of plant cells, issues or organs in the environment with the microorganism-free medium. It uses abilities of the different plant cells to dedifferentiation into stem cells and coming back to the pluripotent status. The extracts obtained this way from the plant stem cells are currently used for production of both common or professional care cosmetics. This work describes exactly impact of the plant stem cell extract, coming from one type of the common apple tree (*Uttwiler Spätlauber*) to human skin as one of the first plant sorts, which are used in cosmetology and esthetic dermatology.

Keywords: stem cells, plant stem cells, pluripotency, *Malus domestica*, *Uttwiler Spätlauber,* plant tissue culture

At the turn of XX and XXI centuries, one of the promising research directions in natural sciences became a regenerative medicine. Researches relating to the different multi-cell organisms abilities in regeneration of damaged organs or even body fragments are carried for years. In some animals, the regeneration processes are intensive and large body fragments may be regenerated (coelenterates, planarians, amphibia), while in others the regeneration processes may be limited only to regeneration of damaged tissue or organs (e.g., human being). In human being, together with the ageing process, which starts about the age of 25 years (chrono-ageing), increases the number of cells in tissues and organs that are subject to a degeneration, and their regeneration processes together with the age subject to slow-down. The stem cells are responsible for regeneration of damaged or wearing-out tissues or organs in human being and in other multi-cell animals (1, 2).

The organ, in which the ageing changes are best visible, is the skin inside which, together with the age the numerous superficial and then deep mimic wrinkles as well as teleangiectasies and melanosis appear. These changes occur both on the epidermal, dermal and subcutaneous tissue levels. Therefore, also the human skin must contain cells responsible for its regeneration (3).

Characteristics of stem cells

The stem cells are the ones able to potentially unlimited number of mitotic divisions and also differentiation to a specific cells. The stem cell division results in appearance the so called progenitor cells, i.e., the partially differentiated cells, which after sequent division shall become the differentiated cells only or they may be subjected to direct differentiation without any further cell division. Depending on what cells appear in result of the stem cell division, and namely if the division is

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symmetric or asymmetric, the role of the given division is defined. If the stem cell is divided symmetrically, and two descendant stem cells appear, then such division is aimed for enlarging the stem cells pool. If the asymmetric division results in appearance of two descendant stem cells that differ: the stem cell and the progenitor cell, then such division is aimed for creation of the line of cells that differ, with simultaneous maintenance of the stem cell line. It's also possible a symmetric stem cell division that however leads to appearing of two progenitor cells. Thus, such division shall result in increasing of the differentiating cells pool, without simultaneous reproduction of the stem line (1).

As mentioned above, the stem or progenitor cells may differentiate into specific cells that form a given tissue or organ. There are also different abilities of the stem cells differentiation (so called potential): from unlimited to limited. Therefore, the stem cells may be divided to (1):

a. Totipotential cells that may differentiate into any organ cell, and also in case of human being into the extra-embryonic structure cells such as fetal membranes. In human being, the totipotential character have the early embryo cells only, where there's no separated embryoblast and trophoblast yet.
b. Pluripotential cells are able to differentiate into the body building structures only, and they can create no extra-embryonic structures such as fetal membranes. In human being, the pluripotential character have, among others, the embryonic cells on the blastocyst stage, and exactly the embryoblast cell in the blastocyst. Thus, the pluripotential cells may differentiate into cells from all the three embryo germ layers: ecto-, endo- or mesoderm.
c. Multipotential cells may differentiate into cells coming, within the embryonic growth, from one germ layer only, thus, from the ecto-, endo- or mesoderm only.
d. Unipotential cells, in turn, are the ones with strongly limited differentiation abilities, thus, differentiating into one particular cell type. The unipotential cells exist in the adult human organs, e.g., in liver, where they are responsible for the given organ regeneration processes.

The other division criterion is the organism type, from which the cell originates. Thus, we can discern the plant or animal stem cells. The latter include the stem cells existing both in the adult human or embryo organisms.

Stem cells of epidermis

The epidermis, being the external body coating, is directly exposed to numerous external environment factors (e.g., mechanical traumas, temperature, pathogenic microorganisms, xenobiotics, UV radiation) that affect the homeostasis disorder not only of the skin, but also the entire organism. Therefore, mechanisms must be created enabling creation of the barrier against factors the action of which shall also speed-up the ageing process. One of such mechanisms is the keratinization process, i.e., keratosis of the epidermis external layers. The epidermis keratinocytes come from its basal layer through the prickle and granular layer (possibly also through the light layer) to the horny layer, inside which due to numerous changes they start to perform into korneocytes. Thus, keratinization means a series of biochemical and morphological changes, being the programmable processes that lead among others, to the cell proliferation ability loss, degeneration of the cell organelle parts (e.g., cell nucleus fragmentation and breakdown, partial breakdown of the endoplasmic reticulum cisterns) and appearing of the new ones (e.g., lamellar bodies, keratohyaline granules), changes in the cell membrane chemical compositions and also appearing of numerous proteins (e.g., involucrine, filagrine binding proteins) and lipids (sterols or phospholipids being the ceramides precursors) (4–6). Researches show that the epidermis granular layer keratinocytes degenerate by apoptosis (3, 7), while the corneocytes located on the horny layer, are subjected to peeling under action of proteolytic enzymes that degrade the corneodesmosomes, i.e., specialized inter-cellular links present in the epidermis horny layer. The ceramides, being the main inter-cellular cement component, are also subjected to changes: degradation to sphingosine and fat acids, which results in the intercellular cement liquefaction (4). Consequently, the corneocytes are mechanically removed from the epidermis surface.

The epidermis regeneration process is possible thanks to the presence of his stem cells in it. Their larger conglomeration is the hair follicle bulge, called the bulge area (3, 8). In this place, such cells intensively divide enlarging its pool, and then they migrate. Part of them migrates to the hair germinal matrix (where they participate in the hair appearing and growth), while the others go to the apical hair part, in order to home the epidermis basal layer. During the migration to the epidermis, the stem cells meet the area located just over the sebaceous gland, where there are concentrated stem cells responsible for production of sebum (8).

However, in order to enable the keratinization and epidermis regeneration process creating the protective layer for the entire organism, many factors must be controlled. Besides the many vitamins, calcium ions, or water level, attention should be paid to a keratinization control process by the keratinocytes themselves. They synthesize and emit many factors that control their process of proliferation and differentiation. They include, among others, EGF (*epidermal growth factor),* KGF (*keratinocyte growth factor)*, TGF-α (*transforming growth factor α*), TGF-β (*transforming growth factor β*) and interleukin 1 (IL-1) (2, 8). These factors, after their release from the cell to inter-cellular space, join the proper receptors on a neighbor (target) cell's membranes and stimulate their proliferation or differentiation (3, 8).

Plant stem cells

The plant stem cells are grouped into niches, called meristems. There are the primary and secondary meristems. The first ones are: apical meristem (stem and root growth cone), intercalary (insert) meristem and germ meristem. The secondary meristems are: lateral ones – cambium (smear) and phellogen and the traumatic (callus) one.

In the sprout top meristem the plant stem cells proliferation and differentiation are controlled by many factors, including the negative reversible loop process between the genes expression products, i.e., *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) proteins. The *WUS* protein is produced by the organization centre cells and it's a signal for the stem cells proliferation, while the *CLV3* protein is secreted by stem cells and it limits the *WUS* expression area. Excess of the stem cells leads to excess of the *CLV3*, which causes reduction of secretion of the *WUS* and it consequently reduces the stem cell proliferation signal. On the other hand, if the stem cell number is too low, then the *CLV3* deficit leads to an increase of the *WUS* protein synthesis, which in turn affects an increase of the stem cell number (9–13).

The root growth cone, on the example of *Arabidopsis* sp., consists of quiescent centre, the cells of which aren't mitotically active. The centre is surrounded with the stem cells producing the distal (cap), lateral (lateral cap cells, epidermis) and proximal (endoderm, primary cortex, axis cylinder) root cells. After a division, one of the cells, directly adjoining the immobile centre, remains the stem cell, while to other loses its connection with the centre and it starts to differentiate. In the roof top meristem, the stem cells proliferation and differentiation are controlled by plant hormones – auxins and probably by some transcription factors, however, these processes aren't fully recognized yet. In the immobile centre, the *WOX5* protein (homologous to *WUS* in the stem growth cone organization centre) is produced, responsible for proper differentiation of the cap cells (9–15).

The traumatic meristem – callus appears in the plant hurting place, it differentiates most frequently with a cambium, however, other tissues are also able to produce it. The phenomenon of callus creation from the differentiated adult plant cells was for first time described in 1902, by the Austrian botanist, Gottlieb Heberlandt. He suggested that the individual plant cell is able to regenerate the entire plant. This experiment was demonstrated in 1958 by cloning of a carrot from the *in vitro* cultivated carrot cells. From this time, many articles appeared dedicated to regeneration of the entire plant from the cultivated cells and/or tissues. The callus creation process is one stage of the somatic embryogenesis (no-fertilization formation of a zygote) – the plant cells are subjected to dedifferentiation and become again the stem cells able to produce a new tissue or even entire organ. The *WUS* protein is responsible for turning back the somatic cells into the stem cells. The researches show that the cytokines are responsible for production of stems from a callus, while auxins are responsible for production of roots (11, 12, 14, 15).

Ability of the differentiated plant stem cells for dedifferentiation back to the pluripotential status are currently used, among others, in elimination of the human skin ageing symptoms, i.e., in production of care preparations or cosmetic procedures.

Plant cell culture technology

The plant cell culture technology consists of many and complicated methods that should ensure growth of plant cells, tissues or organs in the environment with a microbe-free nutrient. The plant cell culture allows synthesis of the biologically active substances that exist in plants, but aren't commonly available in natural environment or are difficult to obtain by chemical synthesis. Such cultures allow access to plant material free from environmental pollutions, microorganisms or toxins, available in every season, with uniform composition, and, first of all, with almost identical content of the active substances in each batch. These aspects were exposed by FAO (United Nation's Food & Agriculture Organization), which as early as in 1994 did propose the plant cell or tissue culture technology as the biotechnological process for pro-

duction of diet supplements. Despite that fact, this technology is still not popular yet and there are few compounds used in cosmetic or pharmaceutical industries, produced using this method, e.g., arbutin obtained from the rose periwinkle (*Catharanthus roseus* L.) used as a whitening agent, safflower and saflorin obtained from coloring safflower (*Carthamus tinctorius* L.) used as a pigment or taxol (paclitaxel) obtained from the western yew (*Taxus brevifolia* Nutt.) being the antitumor medicine (16–19).

The biological bases inside all the above plants are the reservoir of pluripotential stem cells, and also ability of the differentiated cells for dedifferentiation back to the pluripotential status. Any mechanical damage in a plant shall induce appearing of a callus, which consists of the grouped non-differentiated stem cells. In the presence of a proper nutrient the callus may grow in a culture, and using a proper hormonal stimulation it may be stimulated for regeneration of adult plants (so called micro-reproduction technique) (17, 18).

First step in generating of a new plant cell line is to select proper plant material. Next, very important step is sterilization of a tissue, because all the microbes (bacteria, fungi and molds), which may hold or slow-down the culture development, must be eliminated causing no irreversible damage in the meristem cells, necessary for creation of the new cell line. The sterilized plant tissue is reduced to microscopic fragments (called „explants") and placed on the Petrie scale pans including a solid nutrient.

The produced callus is regularly transferred to a proper nutrient including all the substrates necessary for cell metabolism. The culture occurs at dark and therefore the culture looses its photosynthesis ability and it becomes behaving as a heterotrophic organism. At this stage, the culture is supplied with a substance being a source of organic carbon and energy (most frequently a saccharose) and plant hormones (auxin and cytokine), vitamins and also micro- and macroelements. Thanks to the nutrient composition variations we can obtain the cell lines with different properties, from which later on the cell line is selected with the best biochemical and metabolic characteristics (most productive cells with shortest division time). Repeated transfers are made till the moment of obtaining the cell line with the stable and uniform characteristics. It's worthy to point out that the obtained cell line isn't genetically modified, and its selection bases totally on morphological, biochemical characteristics and on the callus tissue growth ability. Fermentation is controlled by a routine measurement of a.o. sugar contents, pH level, cell viability and volume of the produced biomass (16–18).

The essential element of the production process transferred to the industrial scale is adaptation of the selected cell lines for growth in a liquid nutrient that allows significant enlargement of a biomass volume. The suspension cultures require gradual adaptation for growth inside a cone flask (volume ca. 200 mL) and then to growth inside bioreactors that ensure more possibilities (volume up to 100 L). This process is called "scaling". Cultures carried in bioreactors must have ensured a constant temperature and they must be mixed in order to ensure proper gas exchange level required for cell metabolism. The increasing biomass is monitored by measurement of: sugar contents, conductance, pH level, optical density, cell vitality and contents of secondary metabolites such as e.g., ursolic acid (16–18).

Final stage of the fermentation cycle is a biomass processing dependend on the product type and target designation. The first method means a mixing of the culture content in a suspension including liposomes, phenoxyethanol (preservative) and antioxidant (ascorbic acid or tocopherols). Next, the mixture is subjected to a high-pressure homogenization, during which the stem cell walls are demolished, the included components are released and simultaneously the lipophilic components are closed inside liposomes, while the hydrophilic components are dissolved in a water phase. The obtained product is a yellowish and amber color liquid (16). This solution was elaborated and described by a Swiss company Mibelle AG Biochemistry, which did name their technology PhytoCellTec™ (PCT), and the products made by it – PCT™ Malus Domestica, PCT™ Solar Vitis or PCT™ Alp Rose. The other way is a biomass homogenization allowing release of the cell-encapsulated secondary metabolites, and then their extraction and densification. The final product is a powder colored from yellowish to amber and characterizing with a defined active substances quantity. The last way is spreading of the whole stem cells (together with the produced secondary metabolites, polysaccharides complex, phytosteroles and amino acids) in a solid glycerin. The obtained preparation is a liquid with characteristic smell and amber color (16, 19–21). The two solutions are used by an Italian company Instituto di Ricerche Biotecnologiche (IRB) in the technology called High Tech Nature™ (HTN). The preparations with

the exactly defined active substance contents are Teoside™ 10, Dermasyr™, Echigena PluS™ & Echigena 25™. The whole stem cell extracts in a glycerin are named: Echinacea angustifolia stems G™, Leontopodium alpinum stems G™ or Buddleja davidii stems G™.

Uttwiler Spätlauber stem cells

The beneficial apple properties are known for centuries. Apples are cultivated today only for their taste, but earlier the main criterion of the type selection was first of all fruits viability after their picking. One of such apple-tree types is *Uttwiler Spätlauber* growing in Switzerland till today. This is a type cultivated solely due to a possible long-time storage of fruits, which remain fresh even for several months. Some trees come from the quicksets planted in the middle of XVIII century.

Swiss company Mibelle Biochemistry did create the innovative product named PhytoCellTec™ Malus Domestica including the stem cells from the *Uttwiler Spätlauber* apple tree. The tissue, from which the stem cell culture was initiated in external environment, was taken from their fruits (apples). The extract, obtained in a biotechnological process described above, passed a series of tests and researches within the range of anti-ageing action to a human skin and hair (22, 23).

Characteristics of the PhytoCellTec™ Malus Domestica preparation

The *Malus domestica* Borkh. preparation is assigned for use in cosmetics for protection of a face or total body skin. The recommended concentration is 2–5%. The preparation should be entered into the cosmetic water phase by mixing at the temperature not exceeding 40°C. The preparation thermal stability allows its short-time processing at the temperature up to 60°C.

Series of the carried laboratory tests did confirm the preparation efficiency in the range of the human stem cells protection, fibroblast ageing inversion, retardation of the insulated hair follicles and anti-wrinkle action in the "crow's feet" areas. The idea of the PhytoCellTec™ Malus domestica creators was to invent a preparation, which would protect the skin's stem cells vitality, delay their ageing and decrease their effects, and also maintain a healthy skin look and vitality (23).

Protection of the human stem cells

The stem cell extract from *Uttwiler Spätlauber* has been tested in the aspect of ability to maintain alive the stem cells taken from a cord blood. Two test types were carried-out. First experiment was aimed for check of the extract impact to the human stem cell proliferation activity. It have shown that only just 0.1% extract concentration can stimulate growth of the cells multiplication by 80%.

Second experiment was the UV irradiation of the human stem cells. In the preparation, where the human stem cells were placed on the basis without extract, the apoptosis, i.e., programmed cell death was found in more than 40% of cells, while on the basis with 0.1% concentration extract the cell's death was below 10% (22, 23).

Reversal of fibroblasts ageing symptoms

Each cell has a defined life length, which is determined by its divisions number. After such period the cell naturally starts to grow old and it looses its ability to subject sequent divisions. However, the cell ageing may occur earlier if its DNA is damaged. The premature ageing is especially negative if occurs in the stem cells necessary for regeneration of tissues.

The company Mibelle AG Biochemistry carried out an experiment on fibroblast cells, which were subjected to action of H_2O_2. After 2 h exposition, the proper skin fibroblasts did show typical ageing symptoms. After this time, one portion of cells was placed in the 2% extract of the *Uttwiler Spätlauber*, apple tree stem cells, while the other one was placed in the neutral environment and used as a control sample for results comparison. Expression of genes responsible for a cell proliferation and cell growth stimulation was analyzed in two groups with special attention paid to the cell growth and the ageing process with its symptoms. In the control sample the genes were damaged, while the incubation with 2% stem cell extract from *Uttwiler Spätlauber* not only reversed this process, but also stimulated expression of the valid antioxidant enzyme – heme oxygenase 1 (22, 23).

Retarding of the insulated hair follicle ageing

Human hair follicles, being at the anagenic phase, are insulated from skin fragments after a face lifting. Next, they are placed on the growth nutrient, on which they are able to grow in length for more 14 days. After this period, due to no blood flow, the insulated hair follicles are no longer able to grow, because their cells grow old or they pass the apoptosis. Thus, they became a perfect model, on which the company Mibelle Biochemistry shows ability of the *Uttwiler Spätlauber* stem cells for retarding of the tissue atrophy process.

The experiment consisted of location of the one insulated hair follicle portion on the growth nutrient (control sample), while the other on the nutrition with added 0.2% of the stem cell extract. It appeared that the follicles from the control sample become shrink after 14 days of experiment, while the ones located on the nutrient with the plant stem cell extract did grow till 18-th day from their location on the nutrient (22).

Anti-wrinkle action at the „crow's feet" area

The Mibelle AG Biochemistry company carried-out clinical tests lasting 4 week on the group of 20 women aged from 37 to 64 years. The tests were aimed for showing the *in vivo* action of the PhytoCellTec™ Malus domestica preparation included in the cream being the O/W type (oil in water) emulsion. The preparation content in the cream was 2%.

The test included application of the cream, twice a day, on the „crow's feet" area. Depth of wrinkles was measured using the PRIMOS system (optical device for 3D skin surface display), on the test start and after two and four weeks from it. The research showed that the wrinkles became shallow by 8% after two weeks and by 15% after four weeks (22).

Summary

The *Malus domestica* Borkh. apple tree is one of many plant types, on which currently researches are carried enabling use of the plant stem cells in cosmetic preparations. Besides the apple trees, currently they are testing impacts of preparations created using, among others: grapes (*Gamay Teinturier Fréaux)*, alpine rhododendron (*Rhododendron ferrugineum* L.), jasmine gardenia (*Gardenia jasminoides* J. Ellis, *Gardenia augusta* Merr.), common horehound (*Marrubium vulgare* L.), stolonate ground-pine (*Ajuga reptans* L.), lilac (*Syringa vulgaris* L.), alpine edelweiss (*Leontopodium alpinum* Cass.), David's buddleia (*Buddleja davidii* Franch.), narrow-leaved purple coneflower (*Echinacea angustifolia* DC.) or blue cornflower (*Centaurea cyanus* L.). The plant stem cell extracts are invaluable sources of precious active substances. The plant stem cells culture technology, despite possible production of large volumes of active substances, is absolutely environmental friendly and its application enables obtainment of precious compounds, even from the endangered or hardly available plants, causing no unbalance in their natural ecosystem.

The preparations using the plant stem cell extracts may be applied in both cosmetics for everyday care and in the face-mask, serums or procedures carried in beauty salons. The producers define no age limit, however, due to the products properties they shall be rather assigned for complexions with decreased firmness, elasticity and humidity, susceptible ones or for physically active people. The requirements towards contemporary cosmetics and/or cosmeceutics are first of all safety, but also actions giving visible results. The important elements are also the product innovativeness and ecologic advantages. The preparations that include the plant stem cell extracts meet each of these requirements and they perfectly satisfy the XXI-century cosmetology market needs.

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