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Focus on stem cells as cosmetic ingredients

Benefits for human skin and environment

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ABSTRACT: Following the exposure to proper biological signals, adult plant cells have the capability to dedifferentiate and resume multipotent stem, or meristem, cell features. This biological property allows to generate meristem plant cell cultures which offer a technological mean to satisfy many needs of the cosmetic industry. Among these, the major properties are the increased levels of rare phenylpropanoids, which are highly protective for dermal and epidermal cells, and the totally eco-sustainable production process preserving natural biodiversity.

INTRODUCTION

All multicellular organisms, and specifically both plants and animals, start their embryonic development as stem cells. Embryonal stem cells have the capacity to originate, through a number of differentiation steps and functional specialization, all the organs and tissues required for the survival, growth and reproduction in the environment. At the very beginning the stem cells are "totipotent" meaning that each stem cell has the ability to generate a whole organism: two monozygote twins are a typical example. In the differentiated tissue of adult organisms, this ability frequently becomes very limited, or totally lost, to achieve a highly optimized and specialized functional efficiency such as red blood cells and platelets in animals or vessel elements in plants.

Recent scientific discoveries have shown that in many differentiated adult tissues, there are some cells that maintain stem features and are indicated as somatic stem cells. In all known cases, the somatic stem cells are relatively rare undifferentiated cells found in many organs and differentiated tissues with a limited capacity for both self renewal and differentiation. Such cells vary in their differentiation capacity, but it is usually

limited to the cell types in the organ of origin and for this reason they are indicated as multipotent (Figure 1). A typical example are the somatic stem cells of the epidermis that constantly regenerate new hairs. These epidermal stem cells are localized in each hair follicle in a specialized niche known as the bulge area and in physiological conditions are able to generate only hairs, sebaceous glands and epidermis. If the bulge area is damaged, or lost, due to pathological inflammation such as in alopecia areata, then no new hairs can be generated, since the surrounding epidermal cells are irreversibly differentiated and can only produce other epidermis (Jaks et al. 2010; Yang and Costarelli, 2010).

Also plants derive from the growth and differentiation of single totipotent stem cells and, in adult plants, the continuous growth of new tissues is supported by highly active stem cells, technically called meristem cells. In plants the meristem cells are typically located in defined areas of the growing trunk, in the root tips and in the lateral meristem tissues (Kaufmann et al. 2010).

At variance with the animal stem cells, the adult plant stem cells can recover the embryonal totipotency, a process known as dedifferentiation, and thus, when adequate environmental conditions are set, small parts of adult plant tissue can regenerate all the other differentiated tissues of the whole plant. As an example, plant cutting, also known as striking or cloning, is a technique for vegetatively (asexually) propagating plants in which a piece of the source plant containing at least one dedifferentiated meristem cell is placed in a suitable medium. The cutting produces new roots, stems, or both, and thus becomes a new plant independent of the parent.

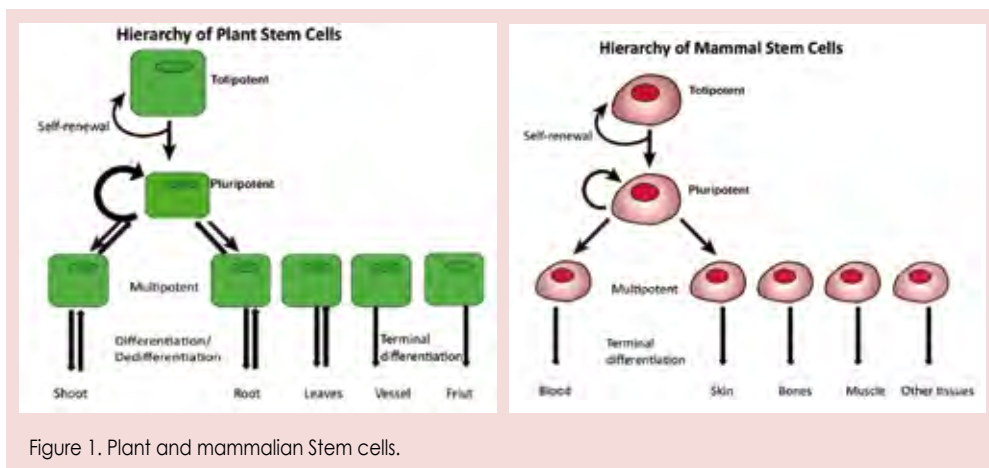


Figure 1. Plant and mammalian Stem cells.

ORGANOGENESIS AND DIFFERENTIATION SIGNALS

Both in animal and plant tissues, the development from a stem cell to structurally differentiated and functionally specialized tissues providing an organ is termed organogenesis. This is a very complex process under control of both soluble and cell bound molecules, that provide external signals informing the cell on its specific location in the organism. As a consequence to the presence of these long range and short range signals, a number of gene expression patterns are coordinately induced in the cell nucleus that commit the totipotent stem cell to differentiate by assuming new structural features and functions and finally form adult organisms. The molecules and the signals that regulate differentiation and organogenesis in animals and plants are very different and specific to each natural kingdom. In plants, the signalling network is mainly based on the presence of auxins, cytokinins (Sablowski R. 2010) and other hormonal substances (such as brassinolide) that diffuse either outside the cells or directly from cell to cell through specialized channels termed plasmodesma. Auxins, such as indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), are plant hormones affecting cell growth by controlling both cell division and cell volume by swelling. Cytokinins, such as kinetin, are phytohormones specifically regulating cell division and, together with auxins, are essential in the growth and organogenesis of plant tissues. Both auxins and cytokinins are required substances present in the growth medium to induce dedifferentiated plant tissue, also known as callus, and to achieve an maintain plant cell cultures from the callus. These properties of phytohormones are essential for the biotechnological applications discussed

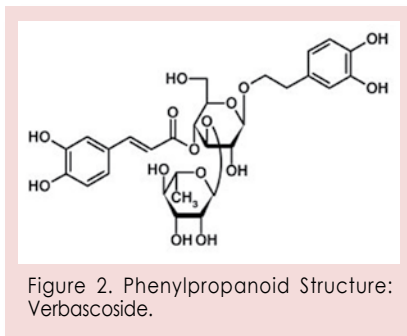


Figure 2. Phenylpropanoid Structure: Verbascoside.

later. On the other hand, the major signalling network supporting organogenesis in animal cells is mainly mediated by soluble growth hormones, such as the Stem Cell Factor (SCF) for blood hemopoietic cells and Insulin-like Growth Factor 1 (IGF-1) and many others (Wu et al. 2010) for cartilage and skin differentiation. Growth hormones play many different functions during organogenesis since, as occurs with SCF, beside cell proliferation they are involved also in cell migration to a final

location. Frequently, this complexity of function is mediated by different structural isoforms of the same molecule generated by RNA alternative splicing and acting either as transmembrane or soluble factors. Besides soluble growth factors, important organogenetic clues are provided also by cell surface bound molecules such as beta-1-integrin and cadherin. As a result of the knowledge available today on this issue, none of the yet identified external biological signals that regulate growth and differentiation are common to both plant and animal stem cells.

Phenylpropanoids: the natural protection for plant and animal cells

Plant meristem cells are rich in substances physiologically part of the cell metabolism and useful to support and protect cell growth. A group of molecules, known as phenylpropanoids (PP), play a very important role in defending the plant meristem cells from the environmental stresses from both biotic (e.g. viruses and other microorganisms) as well as abiotic origin (e.g. UVB and UVA radiations and heavy metal toxicity). As frequently occurs in nature, single molecules are involved in a number of physiological functions and this is true also for PP (Figure 2).

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Figure 3. Plant cell culture technology. A) The sharp border of the plant tissue explant; B) The "callus" outgrowth; C) The plant cell culture; D) The cultured plant cells viewed by phase contrast microscopy.

described, the content of PP monomers is highly reduced in differentiated tissues (roughly the 0.01 percent of the plant dry weight). From this biotransformation of PP to lignin and tannins, derives the difficulty to extract sufficient amounts of purified PP from adult plant tissues to experimentally study and test their biological properties.

However, thanks to the extremely high biological activity of PP on animal cells there is large body of scientific literature on the most abundant PP and namely verbascoside, echinacoside, chlorogenic acid and rosmarinic acid.

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The HTN technology*: plant cell cultures to protect nature

An interesting alternative source of PP is provided by the biotechnological use of plant cell cultures. The plant cell cultures are, in most instances, biologically equivalent to meristem cells and derive from a callogenic tissue or callus. The callus is a wound repair tissue that, in appropriate culture conditions, has been proved to grow for many years, while still maintaining several features typical of meristem cells, including the ability to synthesize high amounts of soluble PP monomers (Figure 3). The technology for the generation of non-GMO cell lines has been widely utilized by botanical specialists for research purposes but, due to high costs of development, it was never utilized for large scale industrial production,

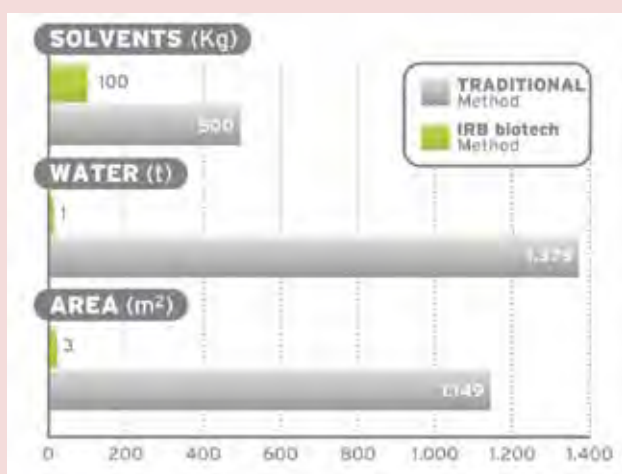


Figure 4. Comparison of land, water and solvent use between traditional and the HTN production technology. Production of 1 kg of pure echinacoside from *Echinacea angustifolia* by traditional methods requires: -three years of soil occupancy before proper maturation (balsamic period) of the roots. This requires an average of 1149 m² of soil and 1379 metric tons of water (either rain or irrigation). Finally, the extraction of the echinacoside from the roots requires also 500 kg of organic solvents. The cell culture technology allows to produce the same amount of echinacoside within 3 m² surface, with 1 metric ton of water, and extract echinacoside with 100 kg of ethanol, which is mainly recycled.

Thanks to the presence of caffeic acid and hydroxytyrosol in the molecular structure of PP, they have a very high antioxidant scavenging activity for reactive oxygen and nitrogen species (ROS and RNS) as well as a strong chelating affinity for metals (e.g. iron and copper) thus removing them from catalyzing dangerous oxidative cycles such as the Fenton reaction. During plant differentiation, PP are initially synthesized as monomers in rapidly proliferating cells. When cell proliferation is reduced or stops, the PP monomers are covalently linked to form polymers such as lignin and tannins which are very compact and highly insoluble substances also contributing to mechanical plant cell protection (Ferrer et al. 2008). Recent studies have demonstrated that several PP, but especially verbascoside, also produce an inhibitory activity on a number of microorganisms, an effect required to defend otherwise unprotected cells (Naoumkina et al. 2010). This turns out to be particularly true for plant meristem cells located at the apical shoot and in the young sprouts and, indeed, the highest level of PP in the plant is detected in the meristem tissues.

The protective action of PP has also been proven for animal cells, and specifically for human skin fibroblasts and keratinocytes and this should not be too surprising since the major environmental stresses (i.e. UV light and environmental toxicants) appear to be the very same for both plant cells and skin cells (Pastore S. 2009). As a consequence of the PP polymerization previously

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with the only exception of taxol production for anti-tumoural treatment. Indeed there are benefits to cosmetics deriving from the use of very active but rare natural substances which are made available almost only by this approach. On the other side the HTN is a fully eco-friendly and sustainable since only a small part of the plant is required to generate the plant cell line and once established, the collection of new plant tissues is not required any more. This greatly reduces soil occupancy, water consumption, pesticide use and solvents to extract low level active substances from large amounts of plant tissue (Figure 4). A further example is available with the *Leontopodium alpinum*, better known as Edelweiss. *Leontopodium alpinum* is a rare and law protected species living in high mountain altitudes, where it is exposed to elevated UV irradiation and extreme temperature shifts. A plant cell line has been generated from a few leaves and now all the PP that are protecting and making this plant survive in its harsh environment are available, with no quantitative limits, for cosmetic use and without collecting any other plant tissue. Since the plant cell culture grows in a completely sterile environment, the final ingredients are totally free of environmental contaminants (pesticides, heavy metals, aflatoxins etc.) and are highly standardized since culture conditions are not modified by seasonal variations thus increasing both safety and quality of cosmetic use.

CONCLUSION

The plant cell cultures provide a unique way to take advantage of highly effective, but rare natural substances, for cosmetic and nutritional use. The HTN technology also provides a non GMO and eco-friendly process to combine and satisfy for large scale production requirements together with low ecological footprint and total safeguard of biodiversity.

REFERENCE AND NOTES

* IRB has developed a original large scale technological approach that provides standardized ingredients for dermo-cosmetic and nutritional applications. **HTN** is the acronym of **H**igh **T**ech **N**ature and implies a mutual beneficial interaction between Technology and Nature.

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