Phytochem Rev (2008) 7:599–605
DOI 10.1007/s11101-007-9082-0

Potential of plant cells in culture for cosmetic application

Cornelia Schürch · Peter Blum · Fred Zülli

Received: 31 August 2007 / Accepted: 22 November 2007 / Published online: 1 December 2007

Abstract Plants and plant derived ingredients are common and of major importance in the fields of pharmacy, food and cosmetics. The cosmetic industry is a fast moving market. Products have short life-cycles and the industry has to come up with innovative products constantly. Most cosmetic products and their applications are defined by active ingredients. These active ingredients may derive from either synthetic sources or from plant sources. Beside this, no other origin like human or animal are accepted or allowed in cosmetics nor are genetically modified plant sources. The whole cosmetic research and development society is therefore desperately seeking for new innovative plant ingredients for cosmetic application. Unfortunately, new plant derived ingredients are limited because several plants of cosmetic interest are not to be used due to following facts: the plants contain toxic metabolites, the plants grow too slow and a seasonal harvesting is not possible, the concentration of plant constituents differ from harvest to harvest or the plant is endangered and not allowed to harvest. With the plant cell culture technology we bring complete new aspects in the development of novel cosmetic plant derived actives. Due to all these findings, we decided to risk the step into plant cell culture derived cosmetic active ingredient production. This article describes the successful establishment of an apple suspension culture producing a high yield of biomass, cultured in disposable, middle-scale bioreactors. The use of a bioactive extract out of these cells for cosmetic application and the efficacy of this extract on mammalian stem cells is also outlined in this article. To obtain a suitable cosmetic product we used the high pressure homogenization technique to decompose the plant cells and release all the beneficial constituents while encapsulating these components at the same time in liquid Nanoparticles. With the plant cell culture technology we bring complete new aspects in the development of novel cosmetic plants derived actives.

Keywords Active ingredient · Large scale production · Suspension culture

C. Schürch (✉) · P. Blum · F. Zülli
Mibelle AG Biochemistry, Bolimattstrasse 1,
5033 Buchs, Switzerland
e-mail: cornelia.schuerch@mibelle.ch

Introduction

Plant extracts and the use of plant parts such as leaves, fruits, flowers, stems, barks, buds and roots are known in cosmetic and pharmaceutical applications since ancient times. Applications of plants and plant extracts in cosmetics are wide spread and where used for purposes such as moisturizing, whitening, tanning, color cosmetics, sunscreen, radical-scavenging, anti-oxidant, immunostimulant, washings, preservatives, thickeners etc. (Blum et al. 2007).

The spectrum of used plants or parts of plants is broad and ranges from algae, succulents, berries, carnivorous plants, herbs, crops, trees etc. A few commonly well known examples of cosmetically used plants are spirula, aloe vera, calendula, ginkgo, ginseng, iris, valerian, sage, lavender, thyme, peppermint, St. john's worth, citrus, peach, guava, avocado, wheat, barley, etc. Countless other applicable plants or parts of plants and their use could be named (Blum et al. 2007).

Although the known benefits, the use of plants or plant materials undergoes certain limitation:

- Availability can be restricted through seasons, limited stock, protection of the plant, problems in cultivation, bad harvest etc.
- Inconstant qualities through seasonal changes, different cultivation methods, geographical differences, other delivery sources, clone types, pollution, physical states etc.

These facts complicate the use of certain plants in a cosmetic application (Blum et al. 2007).

The plant cell culture technology may help to overcome these essential problems in manufacturing cosmetic products, although biotechnology as well as plant cell culture technology is not yet common in the cosmetic field. With cell cultures the dependence of seasonal harvesting has disappeared. There is no concern necessary about endangered plant species. The batch to batch differences in plant components are eliminated.

The other thing is that plant cells in culture are able to produce higher concentrations of specific metabolites (antioxidants or proteins) through eliciting factors like UV radiation, jasmonic acid or toxic substances (Singh 1999). This enables reaching a higher product yield in manufacturing active ingredients. The production of taxol for example could be increased from 350 mg per tree to 300 mg/l through cell culture propagation (Choi et al. 1999).

Cosmetic products and ingredients

Even though the cosmetic field is closely related to the pharmaceutical or food industry, the expectations of cosmetic product consumers and their needs are completely different. They are more sophisticated and are looking for safe cosmetic products that actually do something beneficial to their skin. This is in contrast to the cosmetic legislation which says that only pharmaceutical products are allowed to really act on the body and skin on a systemic way. So the big challenge for the cosmetic industry is to combine these two contradictory needs and to fulfill both requirements. They have to provide functional and safe products. Most of such products are defined by their active ingredients, which also contribute to the marketing position and claims of the cosmetic product. The need of functional, trendy, innovative and safe cosmetic active ingredients is increasing also due to the fact that cosmetic product life cycles are very short. Traditionally, the scientific fields of agriculture, pharmacy and food industry act as innovation sources for cosmetic actives. The transfer of technological and biological know-how from these fields to cosmetics is a natural occurrence (Kostarelos and Rheins 2002). The sources for cosmetic actives to choose from are limited to plant or synthetic sources due to the fact that human or animal sources are objectionable or not allowed, which is also the case with genetically modified plant or bacteria sources. Consequently, all the cosmetic research and development groups are searching for new product ideas and technologies like biotechnology and plant cell culture technology to overcome the industrial, consumer and legislation needs.

Biotechnology and cosmetics

Pharmaceutical products and active substances are often produced by biotechnological processes starting with the cultivation of bacteria, yeast, plant or mammalian cells in large scale bioreactors with a capacity of up to 75,000 l (e.g.: Taxol produced by Phyton Biotech, USA) and going on with the isolation, clarification and concentration of the active substances in a very complex down-streaming procedure. Although it is very widespread in the pharmaceutical industry, biotechnology as well as plant cell culture technology is not yet common in cosmetic field. Most established are ingredients synthesized by or extracted from microorganisms like bacteria, yeast or algae. Such substances include polysaccharides, pigments, amino acids, peptides or proteins. Only a few in vitro processes, based on

plant cell cultures, are already established in the cosmetic industry. Examples for such commercially available products are Shikonine as cosmetic pigment (Fujita and Tabata 1986; Payne et al. 1991) (produced from *Lithospermum erythrorhizon* by Mitsui Petrochemical Industries, Japan), Arbutin as whitening ingredient (Misawa 1994; Yokoyama and Yanaigi (1991) (produced from *Catharanthus roseus* by Mitsui Petrochemical Industries, Japan) or Carthamin as cosmetic pigment (Yamamoto et al. 2002; Haghbeen 2006; Ekiert 2004; Oda 2005) (produced from *Carthamus tinctorius* by Kibun, Japan). Nevertheless, the use of plant cell culture or their metabolite derived cosmetic active ingredients is very rare and in its infant stage. Beside the production of active ingredients biotechnology becomes more and more important in other cosmetic research areas too, like tissue engineering and molecular approaches for product safety and claim testing (Kostarelos and Rheins 2002).

The bridge between biotechnology and cosmetic has already been built and brings new possibilities into cosmetic research including the possibility of genetically individualized cosmetic products based on the science of human genome (Kostarelos and Rheins 2002).

Cosmetics and stem cells

With the recent research on human stem cells (SC) concerning their potential as renewable source of human tissue and their application in many fields of medicine such as gene therapy, organ transplantation, diabetes, reconstructive surgery, cellular therapy etc., SC were also of major interest for cosmetic scientists.

The skin is a multilayer system which renews itself constantly and has the purpose to protect the body against dehydration, injury and infection. In skin, different types of stem cells have been found like epithelial skin stem cells in the basal layer of the epidermis and the hair bulge stem cells localized in hair follicles. 2–7% of all basal layer skin cells are suggested to be stem cells.

Several works have been conducted successfully in the isolation of skin stem cells. Recently it could be shown, that hair bulb stem cells seemed even more capable of differentiating in any type of skin tissue (multipotent) than the epithelial ones (Roh and Lyle 2006; Morasso and Tomic-Canic 2005; Alonso and Fuchs 2003).

Skin stem cells are crucial in sealing, healing, renewing, and growing of both hair and skin. Their capability however can be disturbed by genetic problems, environmental stress or the age of a subject. Furthermore, the life expectation of stem cells is limited through DNA damage, telomere shortening and oxidative stress. Aging of stem cells leads to a decreased capacity of healing, an increased incidence of degenerative diseases, and an increased incidence of cancer in tissues that contain stem cells. The protection and support of stem cells is therefore of great importance.

Use of plant stem cells to protect skin stem cells

Skin stem cells as well as plant stem cells contain evolutionary strongly conserved proteins (100 kDa) which exist in fungus, plants and animals. These proteins interact with nucleotides (both DNA and RNA) and control organ development, stem cell maintenance and tumorigenesis through the regulation of gene expression. This is achieved by chromatin remodeling by DNA methylation (gene silencing), posttranslational modification of histone proteins (e.g. acetylation for transcriptional competence) or mRNA inactivation through micro RNAs (miRNA) or small interfering RNA (siRNA). This enables cells to stably maintain different characteristics despite containing the same genomic material (Epigenetic profiles) (Carmell et al. 2002). Imbalance of these epigenetic factors may lead to abnormal growth, loss of fertility, unusual differentiation of tissue and inducing of cancer. The maintenance and protection of such epigenetic factors in stem cells is crucial for an undisturbed development and functionality of each biological tissue. The components in the cytosol of selected plant stem cells, like dedifferentiated plant cells, are rich in specific substances relevant to protect and maintain these epigenetic factors.

Dedifferentiated plant cells contain a complex matrix of ingredients like salts, acids, phenols, sugars, lipids, proteins and other undefined fractions of compounds which may contribute to a beneficial protecting effect of skin stem cells. It is known from crude plant extracts that better efficacies are exhibited by a complex extract than by separated single

compounds due to synergistic effects. To get similar synergistic effects with plant cell culture extracts the whole cell lysate was used instead of isolated single substances.

Materials and methods

Establishment of a plant cell suspension culture of Malus domesticus

The selection of suitable apple plant material, sterilization of the plant material, callus induction and sub-cultivation on commercially available MS medium had been carried out according to basic standard protocols which can be found in plant cell culture literature (e.g. Plant cell culture: a practical approach, Ed P. A. Dixon, 1994, Oxford University Press). The selection of the appropriate cell line was based on the highest biomass production and shortest doubling time. Establishment of the suspension culture with the chosen cell line in an appropriate liquid media, homogenisation of the suspended cells, sub-cultivation and continuous characterisation of the cell suspension was also carried out according to standard procedures. The fermentation was controlled by routinely checking total sugar content, pH-value, conductivity measurement, cell viability and biomass production using fresh weight biomass.

Up-scaling and biomass production

In a further process the suspension culture had to be scaled up from small laboratory bottles (Erlenmayer flasks, usually about 200 ml volume) to a production scale of 50–100 l in several continuous steps. In this process a fully grown cell suspension culture was used as inoculum with an inoculation volume of 5–20% (preferably 10%) of the next larger culturing volume. For culturing higher volumes than 1 l a special equipped, disposable bioreactor-system, derived from Wave-Biotech AG (Tagelswangen, Switzerland), was used instead of culturing flasks. All higher volume cultivations run at a temperature of 25°C and an aeration rate from about 0.1–0.2 vvm. For different culturing volumes the respective rocking velocities (20 rocks/min) and rocking angles (8.5–9°) were used. Cultivations took place in the dark and the time until a bag had fully grown was about 20 days with an average biomass production of 25 g/l/day. Biomass growth was monitored by indirect measurement of total sugar concentration, conductivity, pH-value and optical density. Cell vitality was also controlled by Evans-Blue coloration and secondary metabolites like ursolic acid and profiles for this cell culture were analyzed by HPLC and UV/VIS measurements.

Down-streaming of biomass

For obtaining an extract of the whole apple cell suspension culture, representing all its beneficial effects, a method with an aqueous and a lipid phase extraction had been chosen. Main constituent of this method is the application of the high pressure homogenisation technology.

The whole cell suspension culture was mixed with 10% of a lipid fraction (Liposomes prepared by Mibelle Biochemistry), 1% phenoxyethanol (Haarmann & Reimer) as preservative and 1% of an antioxidant such as ascorbic acid or tocopherols to further protect the extract from oxidation. After adding all ingredients the mixture was stirred to pre-homogenise and to dissolve the preservative and the other added ingredients. The following high pressure homogenisation at 1,500 bar (Microfluidics) served two purposes: Destruction of the cell membranes to set the extractable ingredients free and generating finely dispersed liposomes (also referred to as nanosomes) which contain the fat soluble ingredients of the cells. This resulted in an aqueous phase containing all the water soluble ingredients and liposomes incorporating the oil soluble ingredients. Huge advantage of this method is that the extract contains all the important compounds of the cells not only the hydrophilic or hydrophobic phase.

In vitro test on SC

The filter sterilized supernatant of the homogenized apple cell suspension culture (PhytoCellTec) served as test product for this in vitro assay on umbilical cord derived mammalian stem cells (MSC). The tests were conducted by BIONET Corp., Taipei 231, Taiwan.

Growth/proliferation test

The cells were seeded on 6-well plates (2×10^4/well) and cultured in a standard medium containing 10% FBS and 0%, 0.01% or 0.1% of the sample. After 24 h of incubation the medium was changed. The cell number was counted after 7 days of incubation. An increase of cell number indicates the stimulation of the MSC and their increased proliferation.

UV exposure damage test

Cells were seeded on 96-well plates (5×10^3/well) and cultured in a standard medium containing 10% FBS and 0%, 0.01% or 0.1% of the sample. The medium was changed after 24 h of incubation. On the second day the cells were exposed to UV radiation. The MTS assay, which shows the viability of the cells and therefore the damage of the UV radiation, was performed 48 h after UV radiation.

Results and discussion

The cultivation parameters of an apple cell suspension culture in a disposable bioreactor system is shown graphically in Fig. 1. The respective measurements of total sugar content, conductivity and pH-value as well as the fresh biomass weight represent a normal cultivation process. Such cultivation lasts about 20 days with a fresh biomass productivity of about 25 g/l/day.

It was not possible to follow a linear up-scaling process although the respective disposable bioreactor system for each scaling step was used. The main problems to deal with while scaling were the volume to surface ratio for aeration and the diffusion-rate of oxygen into the suspension which differed very much from the 1 to 25 l scale. But by optimizing all the incubation factors and the media constituents it was possible to reach a very good yield of biomass during cultivation. It was even possible to reach a fresh biomass yield up to 35 g/l/day, which is enormous regarding the fact, that this culture is not genetically modified. Normally, if a cell culture reaches biomass productivity of about 10 g fresh weight/l/day, it is regarded as commercially interesting. It is not possible to compare these data with other literature data, because most published results deal with the yield of a leading substance or with dry biomass weight not with fresh biomass weight, which was most interesting for our needs however.

The high pressure homogenisation technique provides a very special possibility to damage the cell wall structure in a most gentle way on one hand and on the other hand extracting all the interesting components of the cells in one step. This solves the problem that usually plant cell cultures don't release metabolites into the medium and has the advantage to obtain the aqueous fraction as well as the hydrophobic fraction in one single extract.

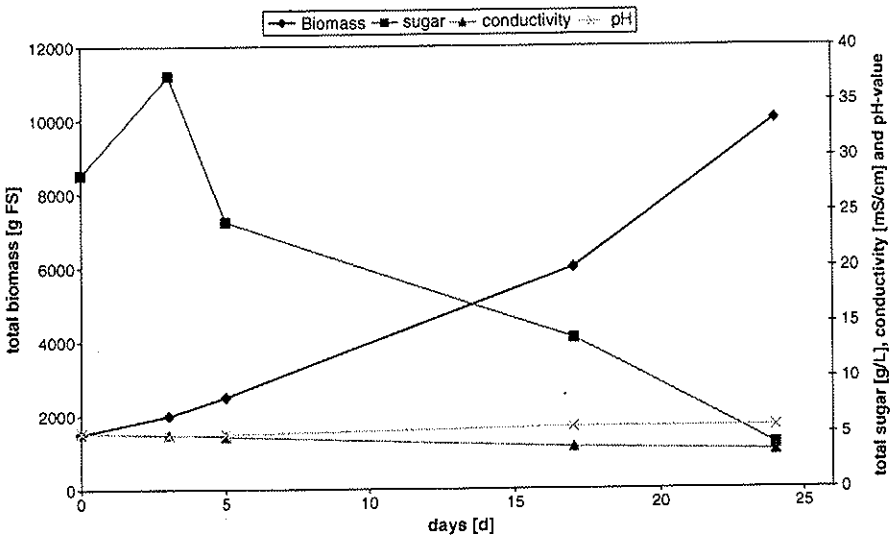

Fig. 1 Growth curve of 25 l apple cell suspension culture

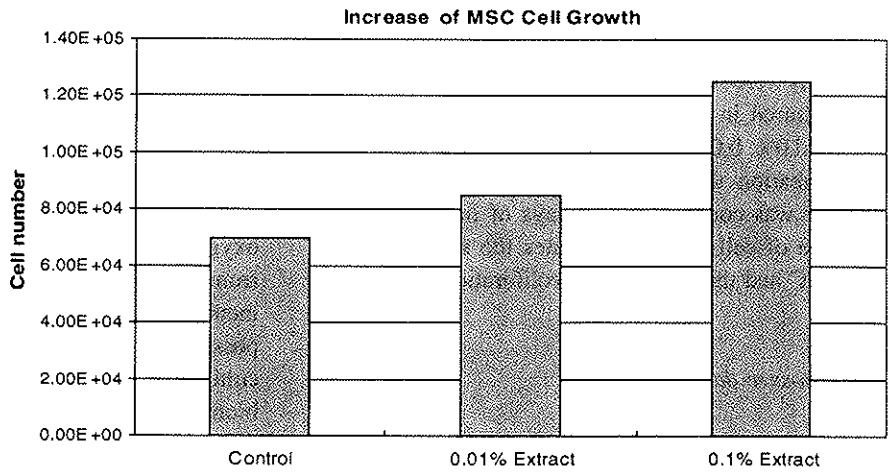

Fig. 2 Increase of MSC Cell Growth with 0.1% and 0.01% apple cell extract

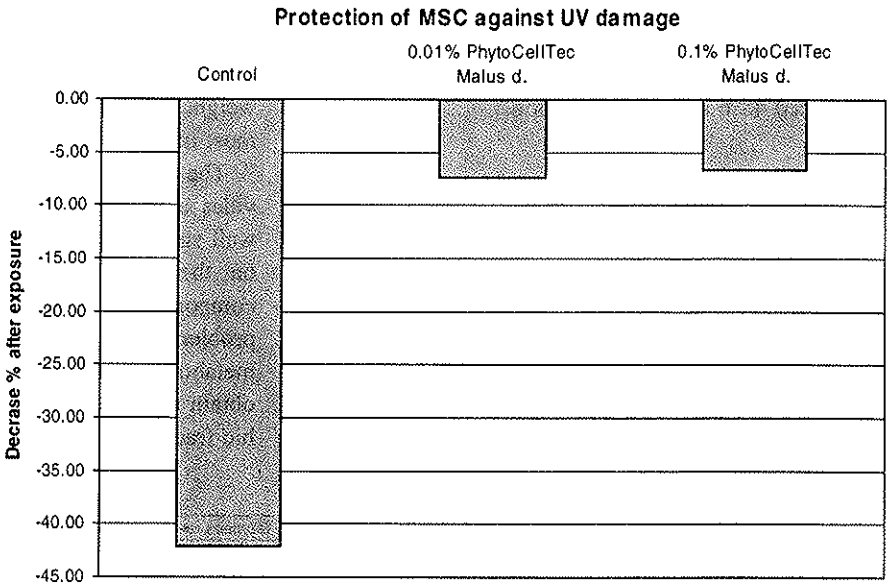

Fig. 3 Protection of MSC against UV damage using 0.1 and 0.01% of apple cell extract

In the conducted efficacy tests the beneficial effects of plant cells on mammalian stem cells were proven. As shown in Fig. 2 the addition of 0.01% and 0.1% of the apple cell extract (PhytoCellTec) to the mammalian stem cells (MSC) resulted in an increase of around 20% and 80% in cell number respectively and thus indicates the stimulation of the MSC proliferation.

The protection of the MSC cells against UV radiation was induced by about 80% using 0.01% of the apple cell extract compared to the experiment conducted without the extract (see Fig. 3).

Conclusion

Plant cell culture technology is an ideal possibility to overcome manufacturing problems with plant materials in terms of availability, sustainability, quality of plant material and secondary metabolites and use of endangered plant species (CITES). For the production of cosmetic active ingredients the use of plant cell technology is only in its infant stage. We succeeded in establishing an apple cell suspension culture for producing cosmetic active ingredients by using a disposable bioreactor system. The average

biomass production of the suspension culture was 25 g/l and per day. The extraction of the suspension culture was carried out using the high pressure homogenization technology to receive a whole cell extract containing all the hydrophilic cell components as well as the hydrophobic constituents. It was possible to show the effect of protection and maintenance on human stem cells with the application of this complex apple cell extract (PhytoCellTec). The cell number of the human stem cells could be increased by 80% with the use of the plant cell extract at a concentration of 0.1%. The integration of 0.01% plant cell extract into the growth medium resulted in a protection of the human stem cells against UV radiation by 80%. With these results the protection, maintenance and propagation of human stem cells by possible epigenetic factors contained in the whole apple cell culture extract could be proven. The positive effects in cosmetic application of such an extract on human skin cells could be predicted.

References

Alonso L, Fuchs E (2003) Colloquium: Stem cells of the skin epithelium. PNAS 100(suppl.1):11830–11835

Blum P, Schürch C, Zülli F (2007) Topische Anwendung von dedifferenzierten Pflanzenzellen für den Schutz und die Erneuerung von Hautstammzellen. Patent pending

Carmell M, Xuan Z, Zhang M et al (2002) The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. Genes Dev 16:2733–2742

Choi H-K et al (1999) Method for mass production of taxol by semi-continuous culture with *Taxus chinensis* cell culture. US Patent 5871979, 16 Feb 1999

Ekiert H (2004) In-vitro Kulturen von Heilpflanzen als potenzielle Quelle für Arbutin. In: Gemeinsame Tagung: Deutsche Gesellschaft für klinische Pharmakologie und Therapie e.V., Gesellschaft für Phytotherapie e.V., Gesellschaft für Arzneipflanzenforschung e.V. Phytopharmaka und Phytotherapie, Berlin, 2004, p 67

Fujita Y, Tabata M (1986) Secondary metabolites from plant cells. In: Proceedings of 6th international congress of plant tissue and cell culture, IAPTC, Minneapolis, p 2

Haghbeen K (2006) Lithospermum officinale callus produces shikalin. Bologia 61(4):463–467

Kostarelos K, Rheins L (2002) Biotechnology impacting cosmetic science: altering the way cosmetics are perceived. Cosmet Toiletries 34

Misawa M (1994) Plant tissue culture: an alternative for production of useful metabolites. FAO Agric Serv Bull 1–87

Morasso M, Tomic-Canic M (2005) Epidermal stem cells; the cradle of epidermal determination, differentiation and wound healing. Biol Cell 97:173–183

Oda H (2005) Utilization of natural colors: photostabilization of safflower (*Carthamus tinctorius* L.) Red Carthamin. Foods Food Ingredients J 210(3):207–213

Payne GF, Bringi V, Prince C, Shuler ML (1991) Plant cell and tissue culture in liquid systems. Hanser, New York, p 346

Roh C, Lyle S (2006) Cutaneous stem cells and wound healing. Pediatr Res 59(4):100–103

Singh G (1999) Elicitation-manipulation and enhancing secondary metabolite production. In: Plant cell and tissue culture for the production of food ingredients. Kluwer Academic, pp 101–111

Yamamoto H, Zhao P, Yakazi K, Inoue K (2002) Regulation of Lithospermic acid B and Shikonin production in Lithospermum erythrorhizon cell suspension cultures. Chem Pharm Bull 50:1086–1090

Yokoyama M, Yanaigi M (1991) In: Komamine A, Misawa M, Dilosmo F (eds) Plant cell cultures in Japan, CMC Co., Tokyo, pp 79–91