P-Cell

Capsicum Annuum Fruit Meristematic Cell Culture

- stimulates cell metabolism making skin more firm
- keratinocytes metabolism and endogenous protein synthesis **+ 105 %**
- skin elasticity **+ 20.5 %**

Abstract

- Red peppers have a significant content of several interesting families of molecules such as capsaicinoids, chlorophylls, carotenoids, polyphenols and flavonoids; we therefore decided to develop a new active ingredient based on stimulated stem cells from red peppers for cosmetic application, called P-CELL.
- Thanks to the data collected in terms of variety selection, dedifferentiation, stimulation processes, stem cell growth rate, chemical composition of the phytocomplex and total antioxidant capacity, a protocol for the production of the red pepper stimulated stem cell culture was developed in order to obtain P-CELL, a new active ingredient for cosmetics based on stimulated stem cells from red peppers.
- During a series of in vitro tests, keratinocytes treated with P-CELL 1% (v/v) doubled their content of protein in just 24 hours. Treated keratinocytes have an extraordinary amount of extra protein so the P-CELL is impressively able to raise the cell metabolism, therefore increasing cell vitality.
- During an in vivo test, skin treated with the formulation containing P-CELL 1% (w/w) showed a net increase of elasticity of 9% in 10 days and of 20% in 20 days with respect to initial values.
- The phytocomplex of the stimulated stem cells contained in P-CELL is able to improve skin tone and compactness by increasing its elasticity. P-CELL is therefore an ideal active ingredient for face and body products aimed at improving skin quality.

Introduction: production of stem cells from red pepper

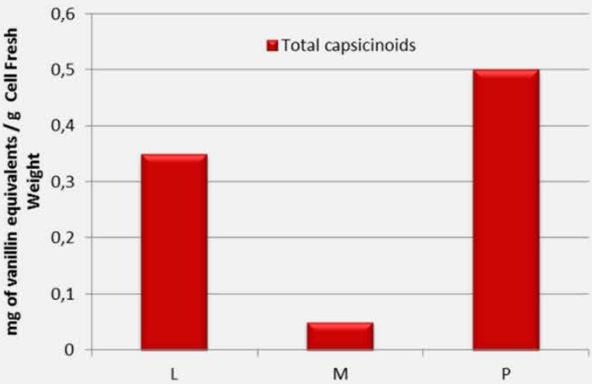
Red pepper or hot pepper (Capsicum spp.) is a native plant of the Americas. It belongs to the Solanaceae family and only 5 species out of the 27 known are domesticated. From these 5 species, hundreds of varieties are cultivated worldwide.

Despite the “fame” of capsaicin, capsaicinoids and other alkaloids, the most representative molecules of Capsicum species, red pepper is able to synthesize several other very interesting molecules (Table 1). Hot pepper is a rich source of vitamins; it contains relevant amounts of vitamin C, vitamin A and vitamins of the B group. It also has a significant fraction of polyphenols, with some interesting anthocyanins and other flavonoids that contribute to the overall antioxidant capacity of red pepper. Finally, this plant has an important content of mineral salts, mainly potassium, calcium and iron.

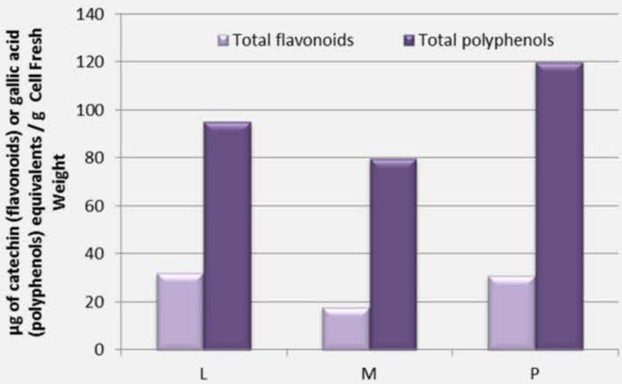
Plant stem cells are undifferentiated cells able to create differentiated cell types. These cells never undergo aging processes and can continuously become any organ, tissue, or cell in the plant, being totipotent cells equipped with amazing regenerative powers.
Plant stem cells are the ideal starting material of cell culture for phytochemical production, being totipotent cells, virtually any chemical that the plant is able to synthesize in any tissue can be produced from these cultures.

To obtain a stem cell culture it is necessary to start from the plant and force some specific tissues to regress into an undifferentiated form to obtain what is called a callus. To obtain the callus used to produce the stem cell culture of P-CELL, three different varieties of red pepper (L, M and P) were selected according to the results of a preliminary literature screening.

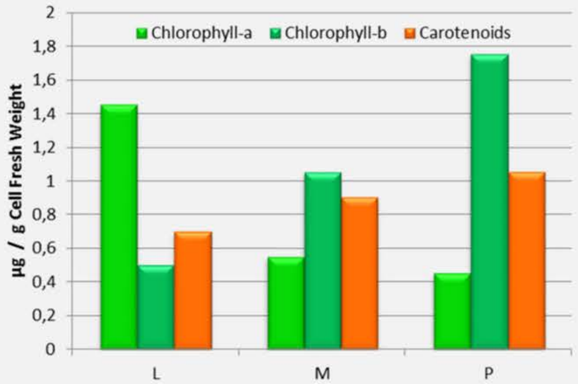
Seeds from the three selected varieties were collected from red pepper fruits and seeded in a solid medium. The seed germination produced red pepper shoots that were used to optimize the solid growth medium used to perform the cell regression to the stem cell. Once all the parameters were optimized in order to obtain a callus from the explants, the three varieties of pepper were used to produce a callus. the callus of each culture was analyzed to evaluate the ability to synthesize chemical compounds of interest for cosmetic applications.



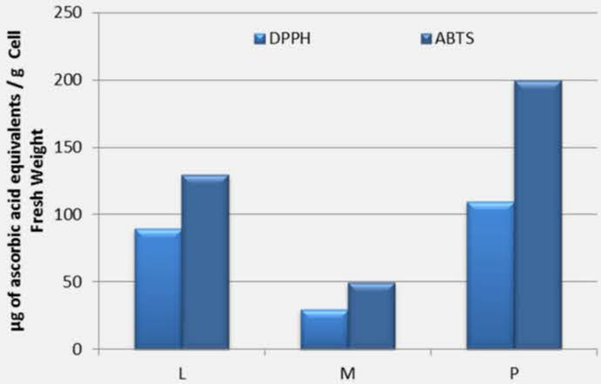
total capsaicinoid content in calluses obtained from different red pepper varieties.



total flavonoid and total polyphenol content in calluses obtained from different red pepper varieties.



chlorophyll-a, chlorophyll-b and carotenoid content in calluses obtained from different red pepper varieties.



antioxidant capacity of calluses obtained from different red pepper varieties.

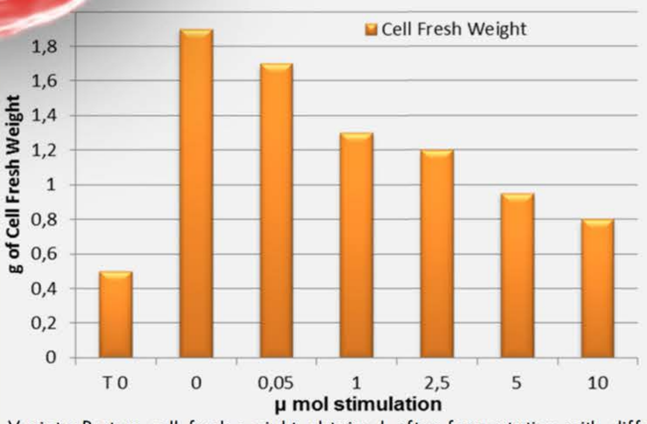
Based on an overall evaluation of the capsaicinoid content, of the values obtained for all the other molecules of the phytocomplex that the callus is able to produce and also considering the antioxidant capacity measured, the variety P was selected for further development in order to obtain a new effective active ingredient for cosmetics, P-CELL.

Stimulation of stem cell culture

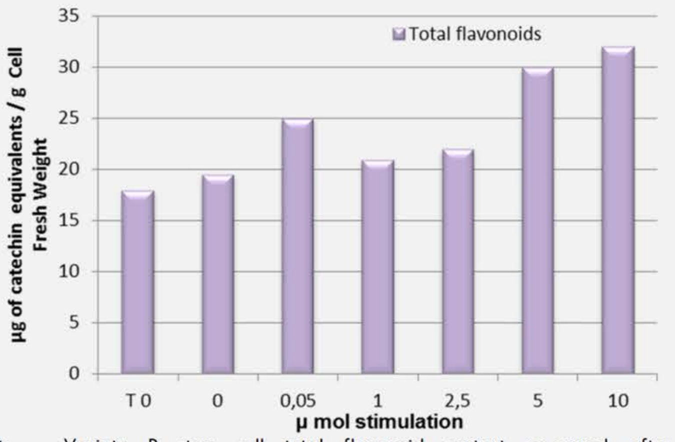
After callus selection, the stem cell cultures were transferred in a liquid medium in order to obtain faster and better cell growth. Furthermore, in a liquid culture it is possible to perform what is called cell stimulation (or elicitation).

This process foresees the introduction in the growth medium of some selected natural molecules at a specific time during fermentation in order to force the vegetal stem cell to overproduce molecules of interest such as those included in the phytocomplex measured during the callus selection.

Thanks to the data collected in terms of stem cell growth rate, chemical composition of the phytocomplex and total antioxidant capacity, a protocol for the development of the red pepper stimulated stem cell cultures was developed in order to obtain the best possible starting material for the development of P-CELL, a new active ingredient for cosmetics based on stimulated stem cells from red pepper.

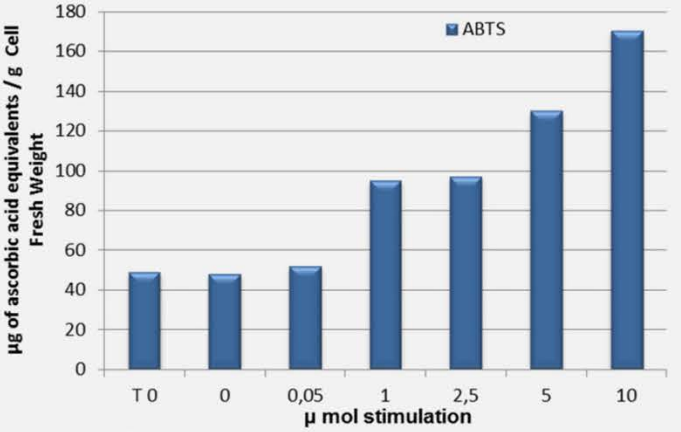


Variety P stem cell fresh weight obtained after fermentation with different concentrations of elicitors. Stimulating molecules (elicitors) are able to force the stem cell to overproduce secondary metabolites, this process often affects cell growth as the stem cells have to use some extra energy in the synthesis of these compounds and are therefore less active in self-replication.



Variety P stem cell total flavonoid content measured after fermentation with different concentrations of elicitors. The content of total flavonoids increases when the concentration of elicitors used during the fermentation is increased

The increase of the total flavonoid content in stem cell cultures treated with different concentrations of elicitors is evident observing the solid matter recovered after the stimulation process. The flavonoids content give the stem cells recovered during the fermentation process a more intense color.

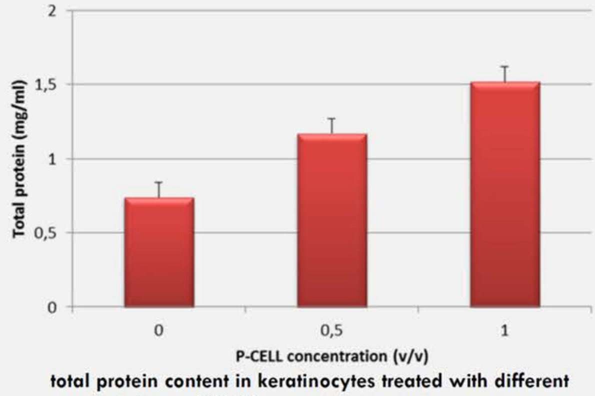


Variety P stem cell total antioxidant capacity measured after fermentation with different concentrations of elicitors. The measurement of the antioxidant capacity of the stimulated stem cell cultures showed that there was a marked increase of the antioxidant capacity when the concentration of elicitors used in the stimulation process rose.

P-CELL: in vitro testing

Several molecules present in the phytocomplex that P-CELL stimulated stem cells are able to produce are well known for their biochemical activities. Due to the complex chemistry that these stimulated stem cells are able to produce we decided to verify whether a cosmetic ingredient containing the prepared culture had any effect on skin cells. In vitro tests were performed on keratinocytes treated with different concentrations of P-CELL in order to see the metabolic effect of this administration. Cells were incubated for 24 hours and the total amount of protein was measured.

The total protein content of keratinocytes increase in a dose dependent manner on increasing the concentration of P-CELL administered to the cells. Keratinocytes treated with P-CELL 1% (v/v) doubled the content of protein in just 24 hours. This is a clear indication that the product has a general stimulus effect of the cell metabolism. The total protein content yields an overall picture of the inner activity of the cell in terms of protein synthesis, the higher the content of protein indicates that the phytocomplex is able to activate keratinocytes, increasing the physiological activity of these important cells of the skin. Treated keratinocytes have an extraordinary amount of extra protein so the P-CELL is impressively able to raise the cell metabolism, therefore increasing cell vitality.



total protein content in keratinocytes treated with different concentrations of P-CELL.

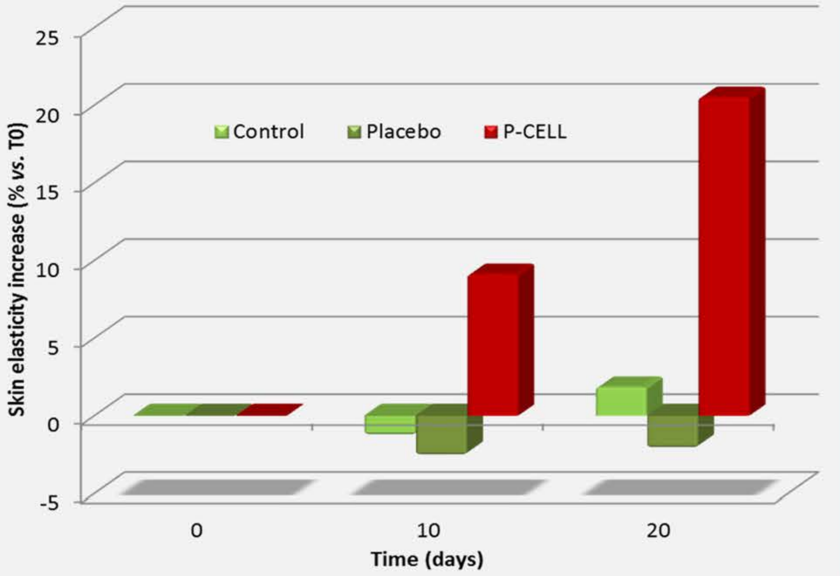
P-CELL: *in vivo* testing

In order to evaluate if the cell vitality improvement observed with the *in vitro* testing can produce any macroscopic effect on the skin, a set of *in vivo* tests was performed. During the tests, the skin health was evaluated in terms of elasticity as a more vital and metabolically active keratinocyte layer is supposed to be more firm and compact, therefore more elastic.

The study was performed on ten healthy volunteers of both sexes aged from 25 to 65. Volunteers were treated on the volar surface of both forearms. Two testing points were present on each arm (for a total of 40 experimental points): one arm was treated with a simple cosmetic formulation containing 1% (w/w) of P-CELL, while on the other arm was used as control and with placebo. The control position was not treated at all while the placebo position was treated with a formulation containing water instead of P-CELL. The product was applied twice per day for 20 days; measurements were collected at T0, and after 10 and 20 days of treatment.

The area treated with the placebo formulation and the untreated area, used as a control, did not show any significant difference between the values measured at time zero and those after 10 and 20 days of treatment. Furthermore, there was no significant difference between the placebo and the control areas both after 10 and 20 days of treatment, as a Student t test yielded a $p>0.30$, meaning that there is no difference between the two areas. These results also indicate that there is no effect due to the cosmetic formulation or due to the application method (a gentle massage) that might have affected the results obtained for the areas treated with the P-CELL stimulated stem cell based product.

The skin treated with the formulation containing P-CELL 1% (w/w) showed a net increase of elasticity. After 10 days of treatment there was an increase of 9% in elasticity if compared with the values at time zero. After 20 days of treatment the skin elasticity was increased by more than 20% with respect to initial values. The statistical significance of the results obtained after 10 and 20 days of P-CELL containing product treatment compared with those of the placebo and control areas was also evaluated.



The data were highly significant, as all the values obtained with a Student t test showed values of $p<0.01$. The data obtained with the *in vivo* testing showed that the phytocomplex of the stimulated stem cells contained in P-CELL is able to improve skin tone and compactness by increasing its elasticity. P-CELL is therefore an ideal active ingredient for face and body cream and lotion aimed at improving skin quality. P-CELL can also be used for specific areas such as the neck/décolleté and the legs/hips/buttocks as an adjuvant in products with more specific cosmetic targets.

CTFA name (requested): Glycerin (and) Capsicum Annuum Fruit Meristematic Cell Culture

P-CELL cosmetic applications

- is an easy to use active ingredient for cosmetic applications.
- is an extremely safe product that can be used for all skin types.
- can be part of any cosmetic formulation containing water, added to cold formulations.

P-CELL can be used in skin care:

- Firming and tensing products;
- Revitalizing and energizing products;
- anti-age products;
- specific area treatments.

Suggested for: face creams, body creams, gels, lotions and tonics, specific area products (lips, neck/décolleté, belly/hips, legs /buttocks), make-up products.

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