

# ENZOGENOL® Skin Cell Research

## Free Radical Defence - UV Protection - Growth Stimulation

Research was carried out to investigate the protective anti-oxidant effects of Enzogenol on oxidative damage in human skin cells.

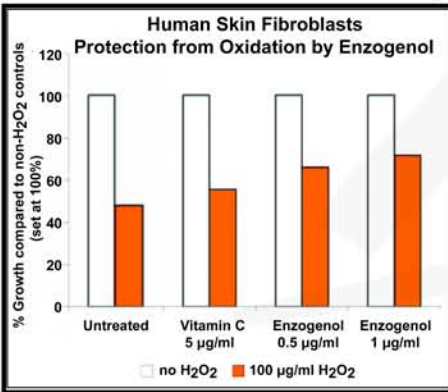
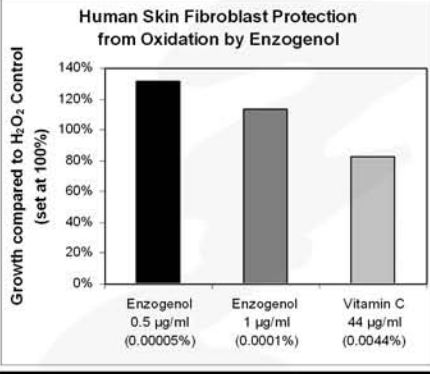
The damaging molecules, that are called Free Radicals, arise either internally as by-products of cell metabolism, or as a consequence of external, environmental factors, such as UV radiation, toxins, cigarette smoke or air pollution. Free radicals oxidise many intracellular and extracellular skin components leading to loss of skin cells and degradation of connective tissue components, like collagen and elastin.

Harmful oxidation by Free Radicals is a major contributor to skin aging. In order to keep the appearance of youthful skin it is critically important to reduce the levels of oxidation. Reduced oxidation will increase the life span of individual skin cells, and protect the integrity of the skin's extracellular matrix of collagen and elastin.

The experiments carried out in this research investigate how Enzogenol protects human skin fibroblast cells from internal and external oxidative damage.

The Results shown here have important implications for Enzogenol's use in external skin applications.

Human fibroblasts (CCL110) were treated for 16 h with Enzogenol® or Vitamin C. The fibroblasts were then challenged with 100 µM hydrogen peroxide (H2O2) for 6 hours, followed by fresh growth medium for additional 48 hours. Cell growth was determined by MTT assay. Shown here is the % increase or decrease in growth compared to the H2O2-treated control cells set to 100%. Hydrogen peroxide treatment leads to growth depression and cell death and is used in this experiment to simulate external oxidative stress for the skin. The results show that Enzogenol® protects the cells resulting in up to 131% of growth compared to the unprotected control cells. Vitamin C, used at 88x higher concentration failed to protect the cells resulting in only 83% growth compared to controls.

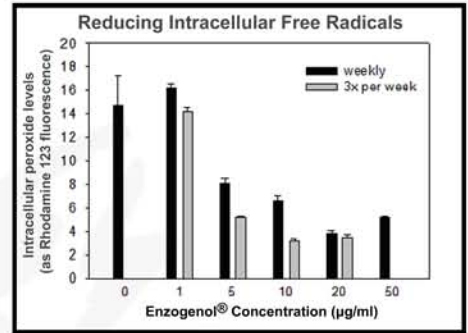
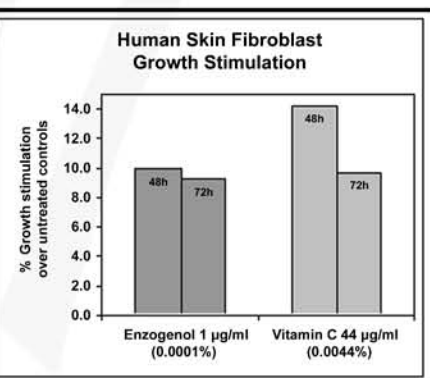


The same experiment was carried out with a lower Vitamin C concentration. Shown to the left is the % growth relative to the controls that were not challenged with hydrogen peroxide set to 100%. Hydrogen peroxide caused severe growth depression to approximately 48% of normal levels. Vitamin C at 5 µg/ml was able to protect the cells to a some degree reducing the growth depression to 55.5%. Enzogenol at 0.5 and 1 µg/ml was more effective and reduced growth depression to 65.7% and 71.4%. This data shows that Enzogenol protects the skin cells 2.3-3 times better than vitamin C at a concentration 5-10 times lower than that of vitamin C. Expressed in concentration equivalents Enzogenol was 15-23 times more effective than vitamin C in protecting skin fibroblasts from oxidation.

Enzogenol® protects the human skin fibroblasts from external oxidative stress. Cells can recover after removal of the oxidant and show up to 131% of growth compared to the unprotected control cells. Enzogenol was 15-23 times more effective than Vitamin C at protecting the skin cells from the external oxidant.

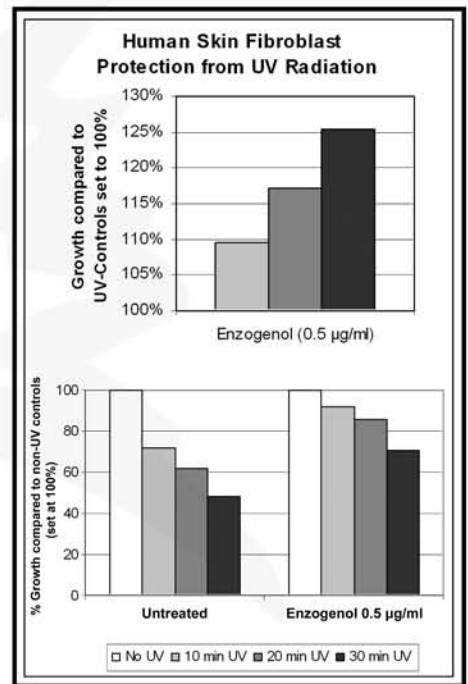
Human fibroblasts (CCL110) were treated for 48 and 72 hours with Enzogenol® (1 µg/ml) or, as a positive control, with Vitamin C (44 µg/ml). Cell growth was determined by MTT assay. Shown here is the % increase in growth over the untreated control cells.

Enzogenol® and Vitamin C stimulate fibroblast growth. Expressed in concentration equivalents Enzogenol® was up to 30 times more effective at stimulating cell growth than Vitamin C.



Human fibroblasts (P43) were treated for 3 weeks with weekly or 3-weekly doses of Enzogenol® at indicated concentrations and accumulation of peroxides in cells was assessed by staining with Dihydrorhodamine123 (DHR) and analysis by flow cytometry. In the living cells the DHR is oxidised by peroxides that otherwise need to be neutralized by the cells antioxidant defence mechanisms.

Enzogenol®, at ≥ 5 µg/ml, reduced the internal peroxide load in the human skin cells by more than 50% demonstrating its potent antioxidant effects in the skin cells. Oxidative stress generated by the normal cellular metabolism is greatly reduced through Enzogenol's Antioxidant Action.



Human fibroblasts (CCL110) were treated for 16 hours with Enzogenol®. The fibroblasts were then challenged with 10, 20 or 30 min of UV radiation. The cells were then given time to recover from the UV challenge by further incubation for 48 hours. Cell growth was determined by MTT assay. Shown at the top is the % increase in growth compared to the respective UV-treated control cells set to 100%. Shown at the bottom is % growth relative to the controls that were not challenged with UV radiation. Results show that UV treatment causes severe growth depression of up to 47.9% of normal levels after 30 min exposure.

These experiments demonstrate the UV-protective effect of Enzogenol® for human skin fibroblasts. Results show that Enzogenol® partially protected the cells from the growth inhibiting effect of UV radiation. Enzogenol was able to reduce UV-induced growth depression from 47.9 to 70.5%; cell growth was 109-125% that of unprotected control cells.