

DC Detoxdefense™

Stimulates skin's own SOD antioxidant protection and detoxification



Multi-Layer Vesicles, concentrated with Cordyceps Sinensis Extract (and) Beta Vulgaris (Beet) Root Extract, deliver powerful skin protective benefits:

- Stimulating skin's own antioxidant protection (SOD)
- Increasing skin's own detoxification mechanism (Cytochrome P450)
- Improving skin integrity by strengthening dermal/epidermal junction (Laminin)
- Protecting collagen I by inhibition of destructive enzyme (MMP8)
- Proven to protect EpiDerm (human skin-like tissue) against UV radiation by 73%



Cordyceps is a fungus, like the mushrooms, reishi and shitake. Cordyceps is a product proven over centuries of use in China, Japan and other Asian countries for maintaining and improving health. However, due to its scarcity, wild Cordyceps has traditionally been available to only the very wealthy. Cordyceps was first mentioned as an anti-aging herb during China's Yin Dynasty around 1700 BC. The first Emperor of the Chin dynasty (259 BC to 210 BC) was known to have paid an ounce of gold for a few days supply of Cordyceps.



Cordyceps is rich in minerals including: Zinc, Potassium, Manganese, Phosphorus, as well as amino acids and vitamins. This extract has the ability to increase the ATP production in the mitochondria of human cells and thus increase overall energy levels. Cordyceps has been shown to increase cellular superoxide dismutase (SOD) for a protective effect. SOD is effective for preventing or decreasing the formation free radicals including superoxide.

DC Detoxdefense is a multi-layer phospholipids vesicle containing cordyceps extract and sugar beet extract offers both defense and detoxifying activities for skin care applications.

Applications: Sun care, Anti-aging skin care, Pollution protection, Detoxifying, Energizing, Spa products

INCI Name: Water (and) Phospholipids (and) Butylene Glycol (and) Cordyceps Sinensis Extract (and) Beta Vulgaris (Beet) Root Extract

Formulation: Use at ph 4-7

Disperse in emulsion or gel at or below 40C

Recommend use level: 2-6%

Distinctive Cosmetic Ingredients, LLC

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DNA Microarray Results (Human Gene Expression)

Objective

The objective of this experiment was to determine whether the results previously obtained DC Detoxdefense in enzymatic and cell-based assays are corroborated by modulation of gene expression in EpiDerm human skin substitute model.

Methods

EpiDerm tissues were obtained from Mattek (Ashland, MA) and cultured according to the manufacturer's instructions. The test materials – Upregulex (Lot# F01071516) and DC Detoxdefense (Product # RON5-7/2, batch# H010705072) - were received on the 2nd of July 2007 and 15th of August, 2007, respectively. DC Detoxdefense at 0.5% (vol:vol). The incubation time with skin tissues was 3 days.

After incubation, skin tissues were harvested, frozen in liquid nitrogen and subjected to RNA extraction with Qiagen kit. The quality of extracted RNA was assayed twice by electrophoresis (after extraction and before microarray analysis) and was determined to be better than control.

Samples were hybridized on Affymetrix chip containing the whole human genome (library HT_HG-U133A). The resulting CEL files were then converted in CHP format using Expression Console software, which were then unlocked in Excel, yielding data on over 22,200 probes (see attached file "Upregulex & DC Detoxdefense DNA microarray profile SBD"). All probes, whose expression changed by 15% or more were flagged.

Results and Discussion

The test was successful in terms that the quality of RNA was outstanding and that the treatment with test materials triggered variation in gene expression pattern in the skin tissue. Upregulex triggered 15% or greater change in the expression of 1087 probes. DC Detoxdefense did it for 1050 probes.

Table below summarizes favorable DC Detoxdefense-induced changes relevant to the skin-relevant enzymatic and cell-based assays.

Probe #	Gene Name	Modulation (%)	Significance
207329_at	Matrix metalloproteinase 8	- 24	Digestion of extracellular matrix proteins
203477_at	collagen, type XV, alpha 1	+15	This chondroitin sulfate proteoglycan is secreted, among others, by fibroblasts endothelial cells and smooth muscle cells, contributes to cell polarity, as it is localized in basement membrane
215078_at	superoxide dismutase 2, mitochondrial	+70	Antioxidant enzyme (SOD) Defense
213519_s_at	laminin alpha2	+19	Laminins are the major non-collagenous component of the basal lamina, such as in epithelium. They are glycoproteins that are an integral part of the structural scaffolding of basement membranes. Laminins are secreted and incorporated into cell-associated extracellular matrices. They are shaped like a cross.
207386_at	Cytochrome P450	+22	Detoxification

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In Vitro Study

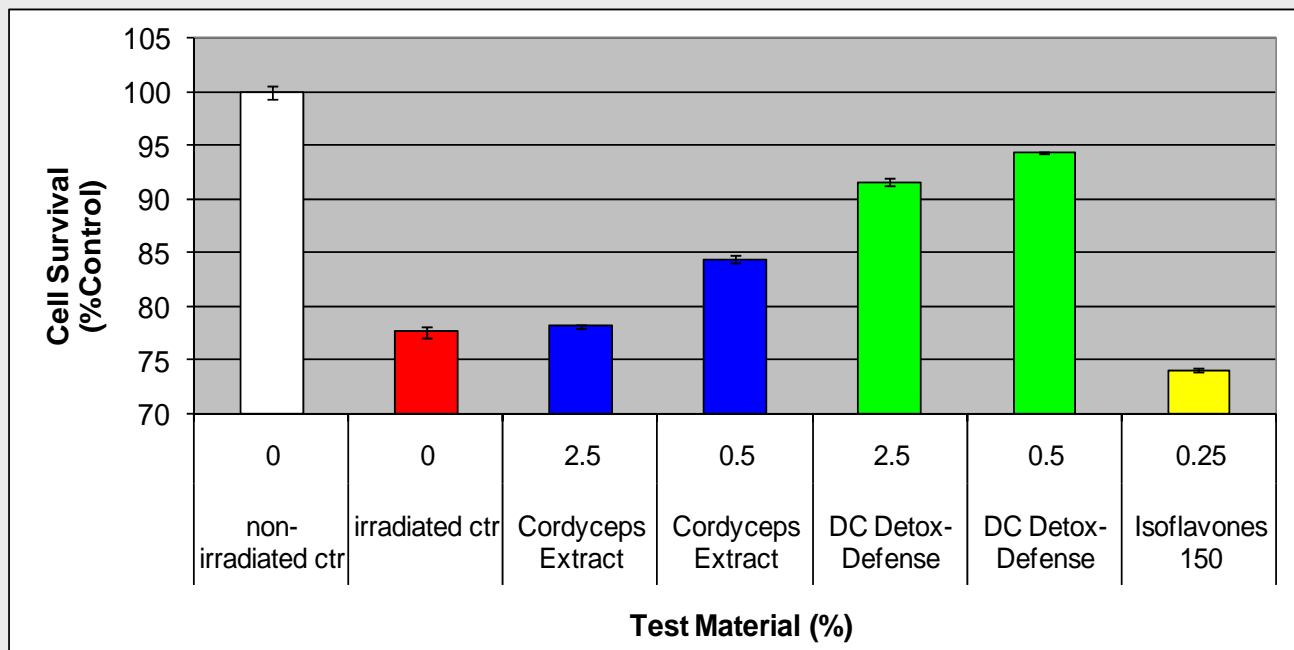
Protection of EpiDerm Tissues from UV Toxicity by DC Detoxdefense

Objective

The objective of this experiment was to determine whether Cordyceps and DC Detox Defense have protective effect against UVB-mediated cytotoxicity in Mattek full thickness skin substitute model.

Methods

EpiDermFT tissues (cat.#EFT 212) were obtained from Mattek Corp. and cultured according to the manufacturer's instructions. The test materials – Isoflavones 150 # 45K/1984/E, Cordyceps Extract and DC Detox Defense - were received on the 15th of May 2008 and kept at 4oC. Isoflavones were tested at 0.25% (w:v), while Cordyceps and Detox Defense at 2.5% and 0.5% (vol:vol). Test materials were incubated with skin substitutes for 48h, then the tissues were UVB irradiated (peak at 302nm) with Hoefer Scientific Instruments transilluminator UVTM-19 for 9min. at 0.8mW/cm2. This UVB fluence was equivalent to one minimal erythema dose (MED) in Fitzpatrick type I-IV human skin, equal to 20-25 min of sun exposure during summer time in Central Park, New York City (Moore et al., 2006). The irradiation was monitored with calibrated radiometer and probe (model S370, United Detector Technology). Following the irradiation, skin substitutes were returned to the incubator for 16 hours, afterwhat cell viability was determined using the standard MTT method and compared to the non irradiated control.



Results and Discussion

As illustrated on the figure above, UVB irradiation of EpiDermFT tissues resulted in the decrease of cell viability (compare the red bar representing the irradiated cells with the white bar representing the non-irradiated cells). Cordyceps extract had a protective effect against this UVB-induced cytotoxicity (up to 27% protection). **DC Detoxdefense** (with same cordyceps concentration as extract) **provided superior protection, up to 73%**. In contrast, the Soy Isoflavones sample at 0.25% had no protective effect in this system.

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