

THE SCIENCE WHITE PAPER SERIES OF IMAGE SKINCARE:

Vitamin C

by Marc A. Ronert MD PhD, Clinical Director Image Skincare

ABSTRACT

Image Skincare offers products with many active, scientifically proven and researched key ingredients to achieve a certain result on the skin. In order to achieve the maximum benefit, not only one key ingredient, but an array of synergistically working ingredients, to target specific skin concerns, is found in every product. This concept is found throughout each and every line and not the name of the product identifies which ingredient is used, but the ingredient listing. All key ingredients are named on the international nomenclature of cosmetic ingredients (INCI) and are furthermore described on product key ingredient manuals. The uniqueness about Image Skincare is the blend of these ingredients into an advanced formulation with a perfectly balanced pH, which dictated the effectiveness of several ingredients. All products follow the concept of the exclusive CPN System™, a unique blending of Correction, Prevention and Nutrition, only offered by Image Skincare. This three in one concept greatly enhances the effect of each product on the skin and achieves results quicker and more profound.

General Findings of Vitamin C

Collagen and Elastin Synthesis

Anti-inflammatory

Photoprotection

Anti-oxidant against ROS (Reactive Oxidative Species)

Ascorbic acid is the chemical name for Vitamin C that is derived from *a-*(meaning no) and *scorbitus* (scurvy), the disease that is caused by a deficiency of vitamin C. It is a sugar acid that can be used for its beneficial properties in normal and aged skin. It is an antioxidant, does up-regulating of neocollagenesis by dermal

fibroblasts, is a cofactor for various hydroxylating enzymes, protects the skin against the effects of ultraviolet A and ultraviolet B radiation, inhibits melanogenesis, stimulates ceramide synthesis, cytokeratin synthesis, and angiostasis. It also helps to prevent cell damage caused by free radicals by acting as a free radical scavenger. It is water-soluble (can dissolve in water) and must be taken in every day as it helps fight infections, heal wounds, and keep tissues healthy. This ingredient helps to prevent and treat aging skin.

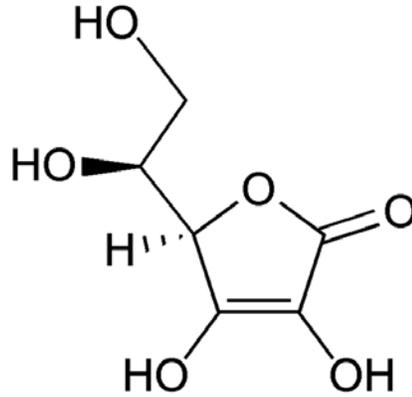


Table 1. Chemical Structure of L- Ascorbic Acid

According to dermatologist assessment, the vitamin C group showed a significant improvement in hydration, small wrinkles, glare, brown spots, roughness, and suppleness of the skin. Also, compared to the placebo, there existed a high increase in the density of microrelief as well as a decrease of deep furrows with the vitamin C over a 6-month period.

Vitamin C compared with placebo treatment for depth of furrows in skin

		T0	3 M o n t h s	6 M o n t h s
Ra	Vitamin C	8.16 μ m	8.15 μ m	6.98 μm
	Placebo	7.73	7.56	7.13 μm

		μ m	μ m	
Rz	Vitamin C	61.74 μ m	62.88 μ m	53.59 μ m
	Placebo	60 μ m	59.22 μ m	55.34 μ m

Table 2: This table shows the Ra (the arithmetic mean of roughness) and Rz (the peak-to-valley mean of roughness) values. There is a greater decrease in deep furrows in skin treated with vitamin C over a 6-month period compared to the placebo.

In the dermis, the vitamin C treated skin showed an increase in elastic fibers compared to the placebo treated skin group. These fibers are what tighten the skin making it look younger and firmer. On the untreated or placebo side there were few elastic fibers that were mostly fragmented electron-dense cores in the papillary and down to the upper reticular dermis. In contrast the treated side was full of several composite elastic fibers that were electron-dense. Along with this collagen bundles were more evenly distributed on the side treated with ascorbic acid. The overall appearance of photodamaged skin was improved in the skin treated with ascorbic acid.

Another study observed the effects of two anhydrous (without water) formulations containing particles of ascorbic acid on neocollagenesis and cytokeatin production in human skin. The exposure time on the skin was 48 hours and microscopy was used to determine results. Neocollagenesis production or the production of new collagen leads to firmer and younger looking skin. Cytokeatin production is a production of proteins your skin needs that also leads to firm and tight skin. As you age these processes slow down. This study found the result of an application of ascorbic acid treatment to the skin is new collagen formation and increased production of cytokeatin.

Novel Forms of Vitamin C: BV-OSC

There are different forms of Vitamin C which differ from stability and effectiveness. Image Skincare uses a blend of these types to

achieve increased absorption and effectiveness. The first form is the active form, L-ascorbic acid, which is unstable when it comes to contact with air. More stable forms are esterified derivatives, such as Ascorbyl Palmitate and Magnesium Ascorbyl Phosphate, which is the most stable of these three. Recently, more advanced, modified and more stable forms are available and introduced to Image Skincare products. Key factor to search for novel forms of ascorbic acid is the bioavailability- the ability of the body to utilize and absorb a vitamin or nutrient.

A unique, oil-soluble form is BV-OSC, Tetrahexydyl-Ascorbate.

Some benefits of BV-OSC:

- Anti-Oxidant activity, inhibiting lipid peroxidation
- Whitening Effect- clarifying and brightening activity, inhibiting melanogenesis. Provides a more even skin tone.
- MMP Inhibition (reduction in free radicals)
- Collagen Synthesis- stimulation of collagen production
- Collagen Protection

Pure Vitamin C is very unstable. It is sensitive to oxidation and gives finished formulas a yellowish/ brownish tint. Pure Vitamin C is not the most active form for collagen synthesis and anti-oxidation.

BV-OSC offers a stable, oil-soluble Vitamin C derivative.

The penetration of Ascorbic Acid is very limited compared to BV-OSC.
BV-OSC maintains a higher penetration rate even when the Ascorbic Acid is increase by 25 times that of BV-OSC.

BV-OSC protects against cell damage: prevention of UV-B & UV-A damage

BV-OSC has an excellent penetration in keratinocytes. As a result the cytoprotection against UV-B is increased. The cell viability is increased up to 30% when BV-OSC is applied compared to pure Vitamin C. BV-OSC reduces UV-B Damage.

BV-OSC inhibits the release of 8-OHdG ((8-hydroxy-2'-deoxyguanosine) the quantity released measures UV-A damage in the skin)

BV-OSC protects the cell against UV-A damage

BV-OSC has anti-aging properties: Collagen Synthesis

Adding the same amount of BV-OSC to Fibroblast culture- the proliferation of the cells was increased by 50%. The fibroblasts significantly increased

collagen synthesis. The same dosage of ascorbic acid increases collagen synthesis by only 25%.

BV-OSC has MMP Inhibition Effect and whitening properties.

One of the many benefits of vitamin C in cosmetic formulations is the ability to provide a more even skin tone: “clarifying and brightening” effect.

In vitro test shows that BV-OSC reduces melanogenesis by more than 80% (see attached literature pg. 18)

BV-OSC can also be used to treat Acne and is contained in the Clear Cell medicated acne lotion.

For more information about BV-OSC used in Image Skincare products please refer to attached literature. The ingredient is listed under Ascorbic acid in our formulations.

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Presents

BV-OSC **(TETRAHEXYLDECYL ASCORBATE)**



A STABLE, OIL-SOLUBLE FORM OF **VITAMIN C**

ANTI-OXIDANT

WHITENING

COLLAGEN SYNTHESIS

UV PROTECTION

MMP INHIBITION

COLLAGEN PROTECTION

DNA PROTECTION

NEW DATA: COMET ASSAY

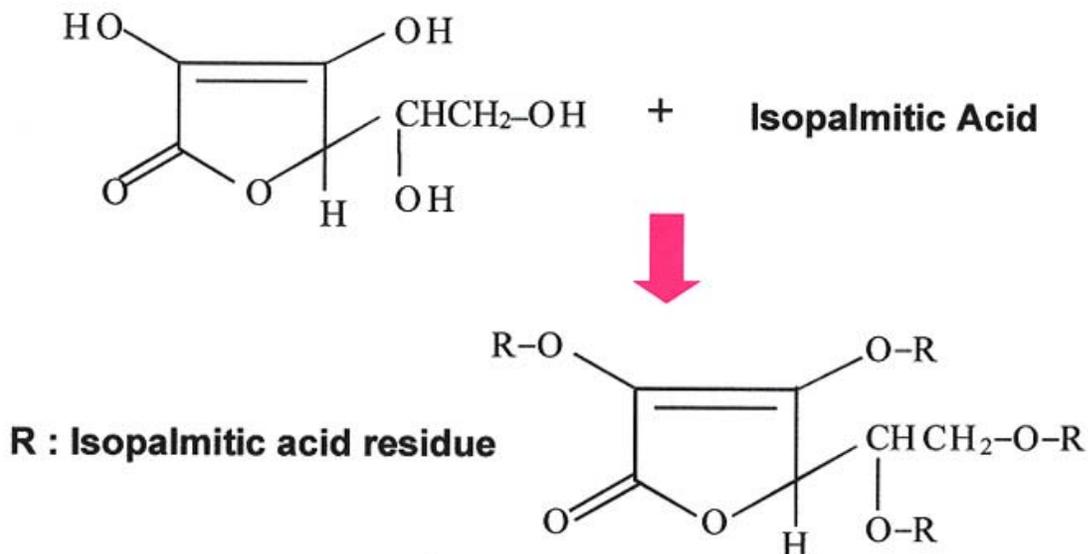
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BV-OSC

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INTRODUCTION

Development of New Oil Soluble Vitamin C Derivative



INCI Name : Tetrahexyldecyl Ascorbate
(BV-OSC)

BV-OSC is a stable, oil-soluble Vitamin C ester.

The benefits of using Vitamin C in formulations include:

- Anti-oxidant activity, inhibiting lipid peroxidation
- UV-A and UV-B protection
- Clarifying and brightening activity, inhibiting melanogenesis
- Stimulation of collagen production
- Inhibition of MMP's

Pure Vitamin C is very unstable. It is sensitive to oxidation and gives finished formulas a yellowish tint. Note also that pure vitamin C is not the most active form for collagen synthesis and anti-oxidation.

Barnet Products offers a stable, oil-soluble Vitamin C derivative:

---BV-OSC

INCI NAME: Tetrahexyldecyl Ascorbate

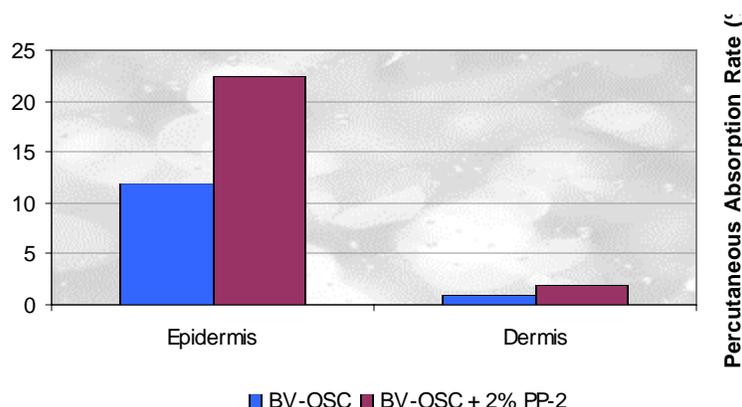
1. BV-OSC is very bio-available.

A. Percutaneous Absorption of BV-OSC and Delivery and Deposition with Polyolprepolymer-2 (PPG-12/SMDI Copolymer)

The first part of this presentation demonstrates that BV-OSC is retained in the epidermis and, to some extent, in the dermis. This retention can be doubled with the use of 2% PP-2 (Figure 1). A cream containing 5 μ M of BV-OSC was applied on the skin set on Franz. diffusion cells. BV-OSC concentrations in the epidermis and dermis were determined after 24 hours.

The second part of the presentation compares the penetration of “equivalent Ascorbic Acid” into the epidermis of BV-OSC and VC-PMG. Results show equivalent Ascorbic Acid penetration with 0.75% BV-OSC and 3% VC-PMG, suggesting that BV-OSC provides excellent penetration (Figure 2).

Figure 1: Excellent Percutaneous Absorption



Method of Measurement: Diffusion cell with human skin

Figure 2: Amount of Ascorbic Acid Penetrated Into the Epidermis

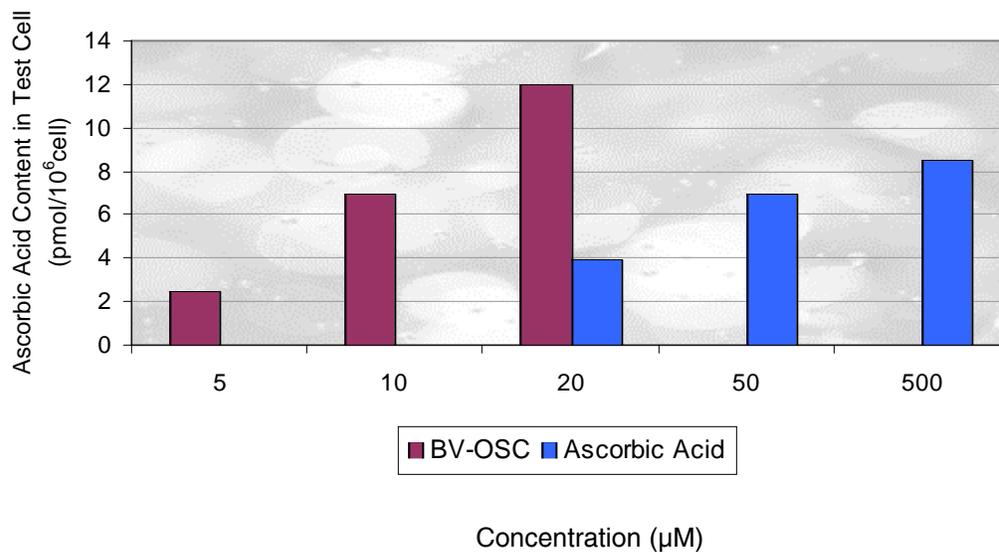
	VC-PMG	BV-OSC
Molecular Weight	289.5	1129.8
Ascorbic Acid Moiety in the Molecule	59.4%	15.2%
Skin Penetration (Amount in Epidermis)	0.7%	11.6%
Ascorbic Acid Penetrated into the Epidermis	0.42%	1.76%
Example In the Formulation	3% VC-PMG 0.0126%	1% BV-OSC 0.0176%

Tested on excised human skin with the addition of 3% Polyolprepolymer-2 from Bertek using radiolabelled samples on diffusion cells.

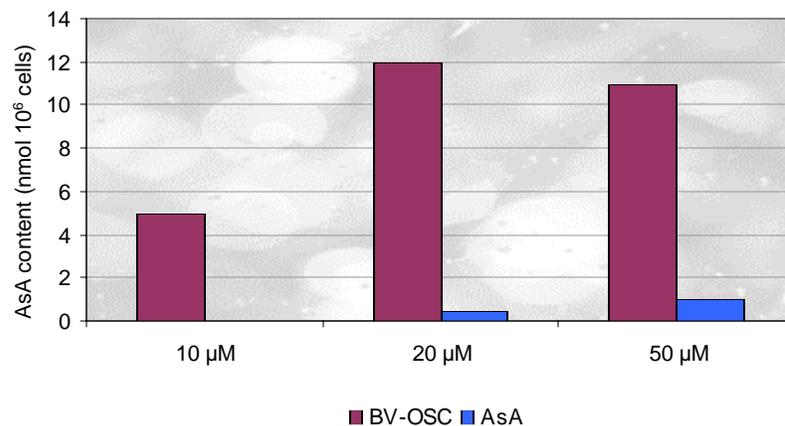
B. The penetration of Ascorbic Acid is very limited. The difference in penetration at levels between 50 μM and 500 μM is minimal.

The penetration of BV-OSC is dose-dependent, and surpasses that of Ascorbic Acid at the same concentration (20 μM) by three-fold. BV-OSC maintains a higher penetration rate even when the Ascorbic acid is increased by 25 times that of BV-OSC.

Uptaken Content of Intracellular (Keratinocytes) Ascorbic Acid



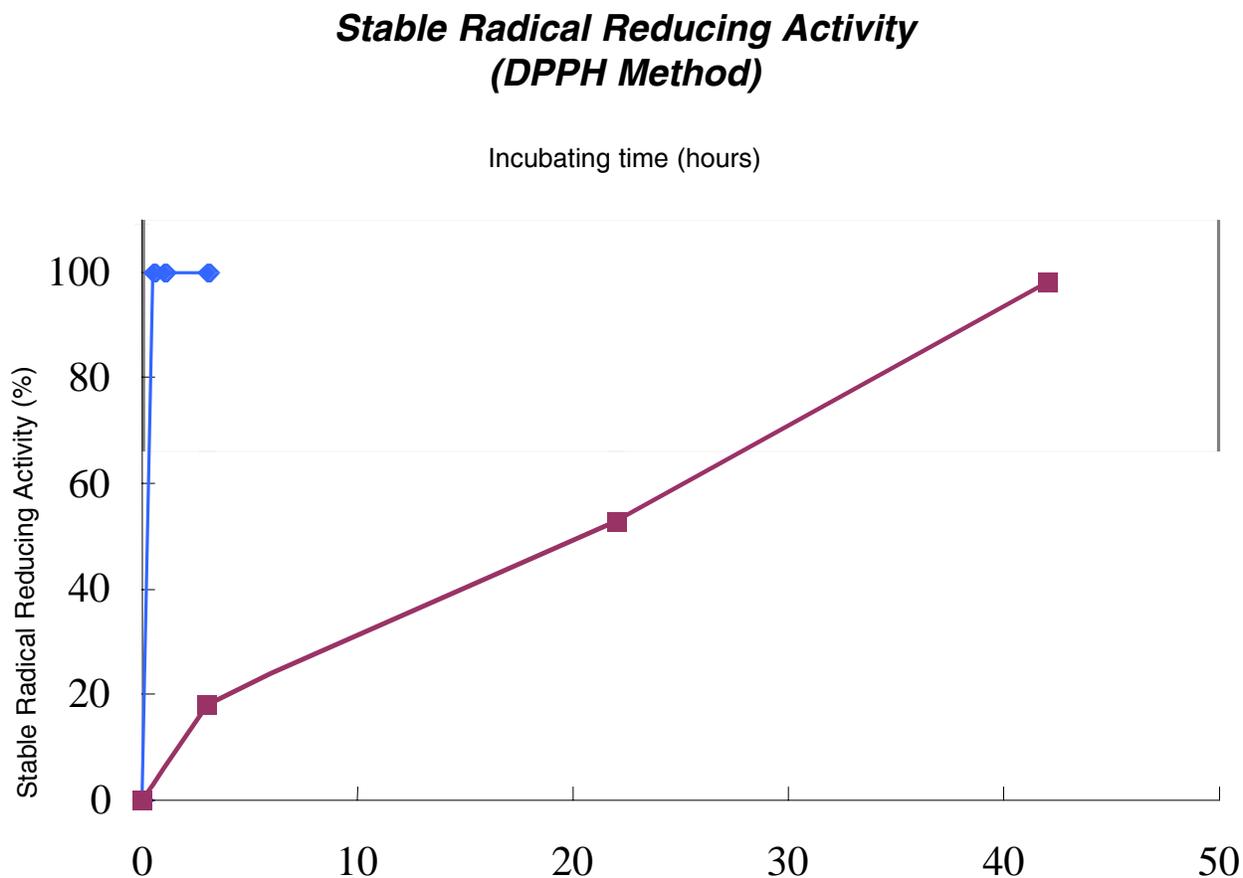
Fibroblast



Cells were treated with the medium containing various concentrations of BV-OSC or Ascorbic Acid (AsA). After 2 hours in incubation, cells were homogenized and the content of free Ascorbic Acid was determined using HPLC.

2. BV-OSC is very functional for stress protection.

A. Anti-Oxidant Activity of BV-OSC



The reducing activity of each 2.0mmol of BV-OSC (in red above) or Ascorbic Acid (blue) was measured by using a stable radical DPPH (0.01mmol) with phosphate buffer (pH 7.0) at 37° C for 48 hours.

As shown, for BV-OSC, the reduction ratio (%) of DPPH after 3 hours, 24 hours and 42 hours from the reaction started was 18.7%, 52% and 98.1%, respectively.

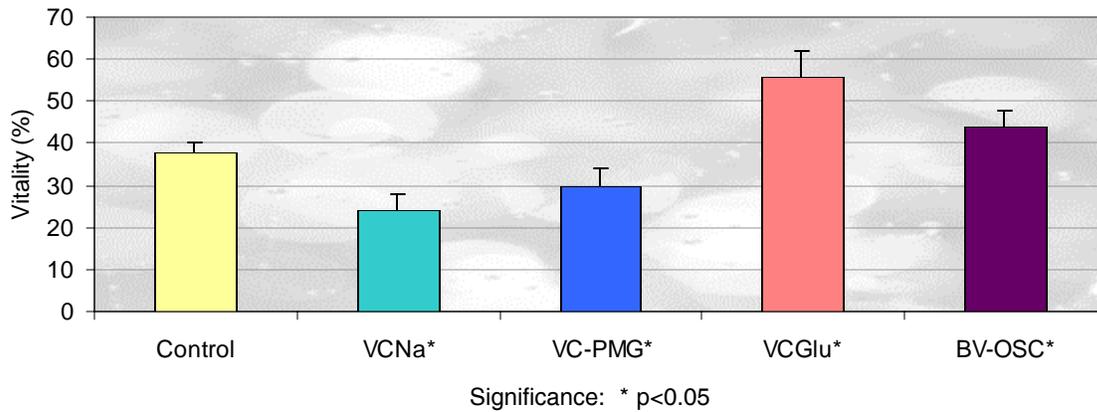
On the other hand, for Ascorbic Acid, the reduction ratio (%) of DPPH reached almost 100% after 30 minutes. The difference of the reducing activity between BV-OSC and Ascorbic Acid seems to be related to the difference of activity of the 2-hydroxyl group in the structure which possesses the proton donating ability.

2-hydroxyl group in BV-OSC is blocked with 2-hexadecanoyl moiety. In order that BV-OSC possesses the reducing activity against DPPH, it is necessary to hydrolyze the 2-acyl moiety and liberate the 2-hydroxyl group.

Accordingly, BV-OSC seems to act as a radical scavenger more slowly than Ascorbic Acid.

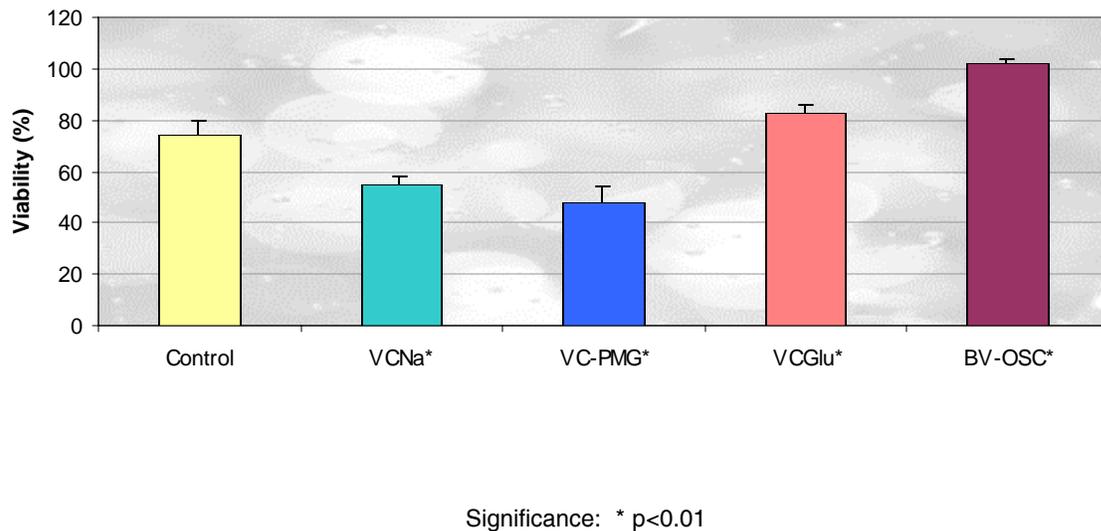
B. BV-OSC Protects Against Cell Damage

Protection of Cell Damage Induced by H₂O₂



HaCaT keratinocytes were treated with various 100 μ M of various Vitamin C derivatives for 24 hours. After treatment of 20 μ M for 2 hours, cell survival was estimated.

Protection of Cell Damage Induced by t-BHP

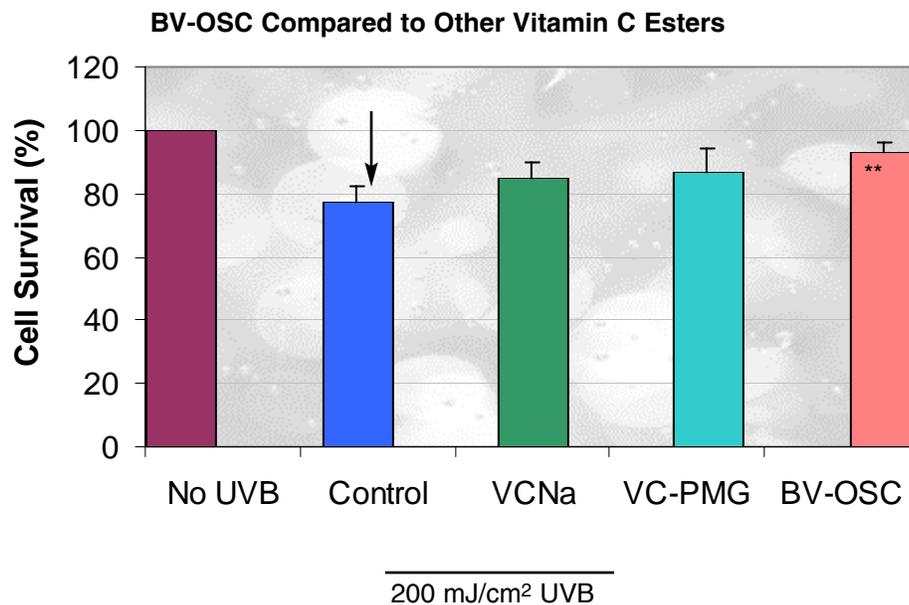
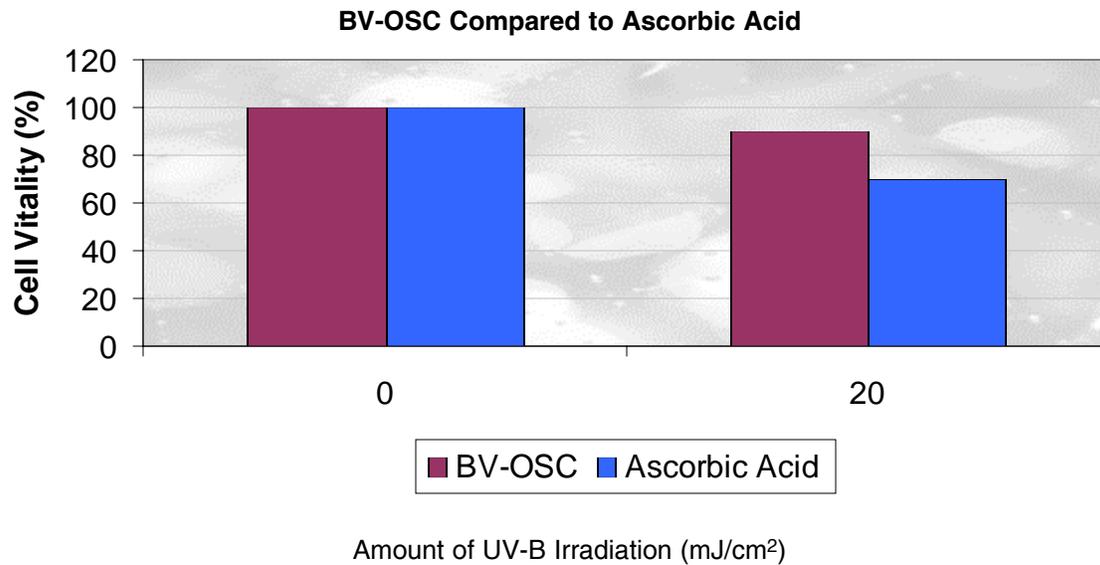


HaCaT keratinocytes were treated with various 100 μ M of various Vitamin C derivatives for 24 hours. After treatment of 1.0 nM of t-BHP for 4 hours, cell survival was estimated.

C. Prevention of UV-B Damage with BV-OSC or Ascorbic Acid

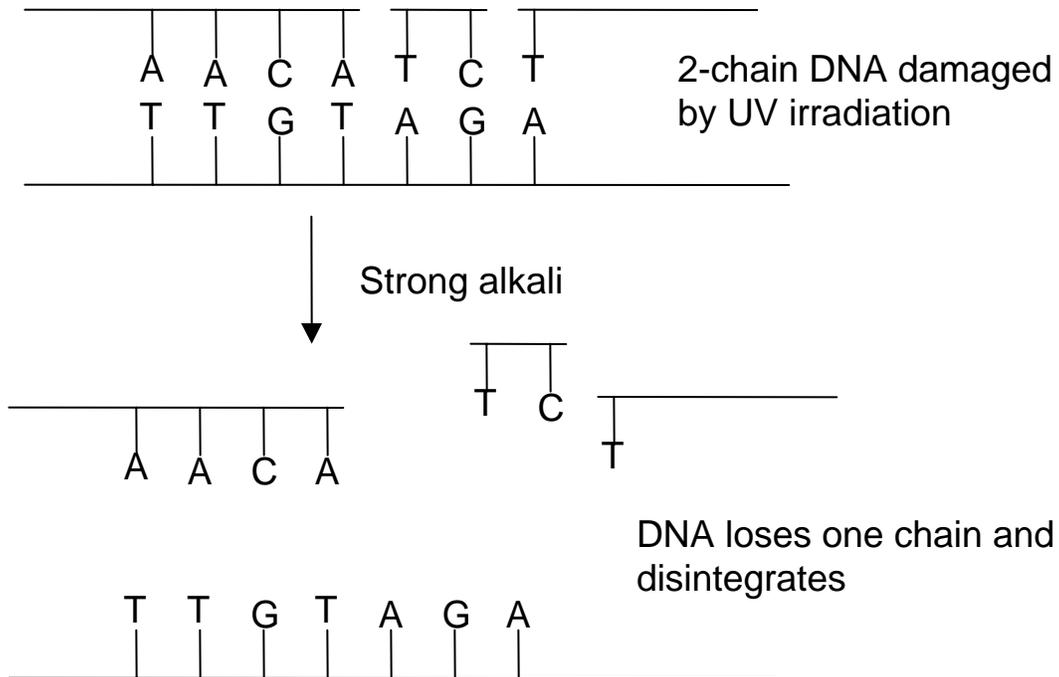
BV-OSC has an excellent penetration in keratinocytes. As a result the cytoprotection against UV-B is increased. The cell viability is increased up to 30% when BV-OSC is applied compared to pure Vitamin C.

Cytoprotective Effect Against Cell Mortality of UV-B Irradiated Skin Keratinocytes



HaCaT keratinocytes were treated with various 100 μM of various Vitamin C derivatives for 24 h. After 24 h from UVB irradiation, cell survival was estimated. Significance: * p<0.05, ** p<0.01.

→ **Comet Assay**
Idea



What is Comet Assay ?

The comet assay, also called the 'Single Cell Gel Assay', is the technique to detect DNA damage and repair at the level of single cells. The comet assay or single cell gel electrophoresis assay is based on the alkaline lysis of labile DNA at sites of damage. 'Comet Assay' is one of the most popular tests of DNA damage detection (e.g., single- and double-strand breaks, oxidative-induced base damage, and DNA-DNA/DNA-protein cross linking) by electrophoresis, developed in recent years.

Merits Of Comet Assay :

- Very high sensitivity to detect DNA damage
- Rapid and easy to handle
- Little amount of cell samples needed
- Applied to most eukaryotic cells

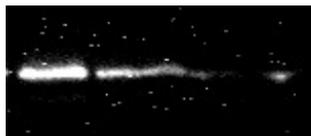
Western Blot (p53 expression suppressed by BV-OSC)

Result:

BV-OSC @ 0.005% : p53 expression decreased to 50%

BV-OSC @ 0.01% : p53 expression decreased to 10%

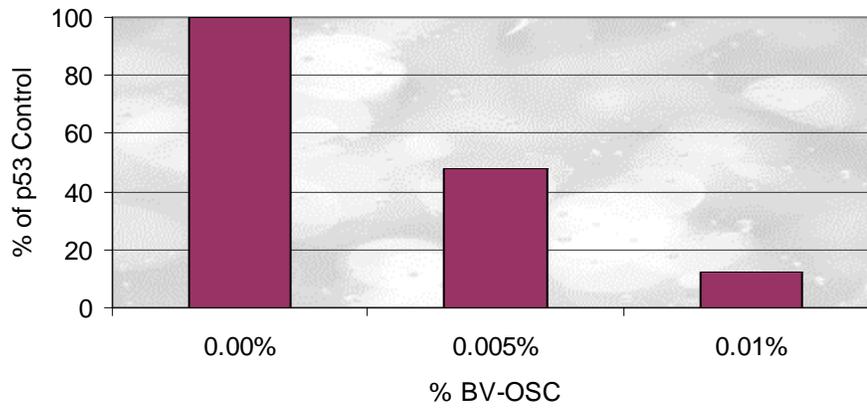
Concentration of BV-OSC:



0 0.005 0.01 (%)

→ **Quantitative Evaluation of Protection**

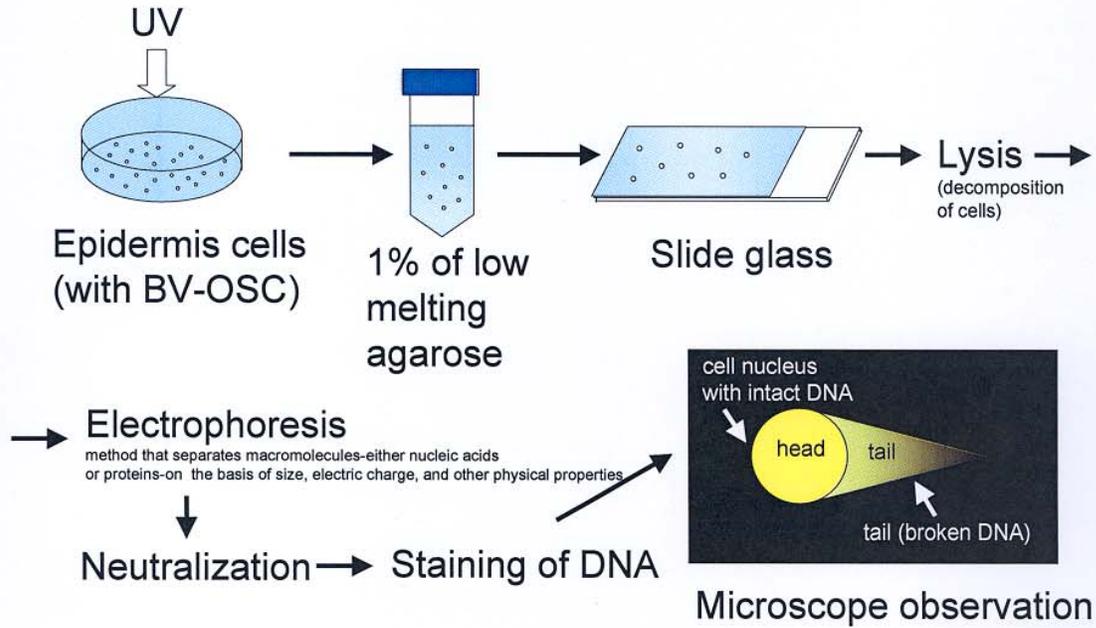
UVB Induces p53 Synthesis



Human dermal fibroblasts were treated with various concentration of BV-OSC for 24 h. 100 mJ/cm² UVB was irradiated following additional 24h cultivation. p53 (proteins that cause apoptosis, or cell death) is secreted in the cell. The cells were then lysed and the medium was tested for p53 expression by Western blotting.

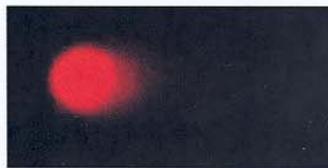
BV-OSC limits p53 synthesis. It reduces UV-B damage.

Test method



Suppression of DNA damage induced by UVB

Control



UVB 10mJ/cm²

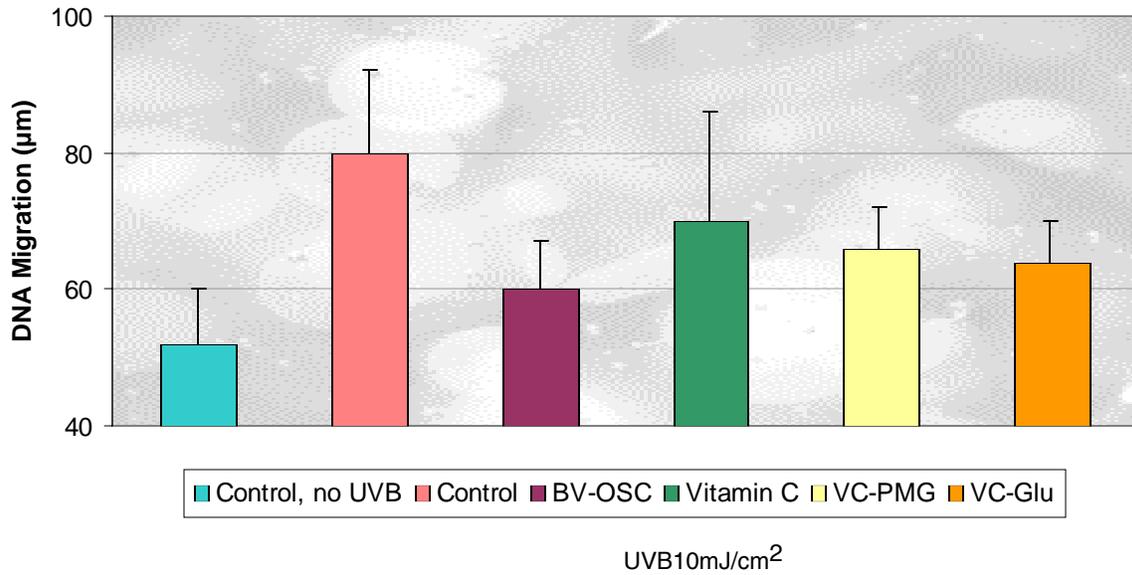


UVB 10mJ/cm² +
BV-OSC (100 mM)



DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with VC derivatives for 24 h, were exposed to UVB at 100 mJ/cm². Cells are stained with etidium bromide.

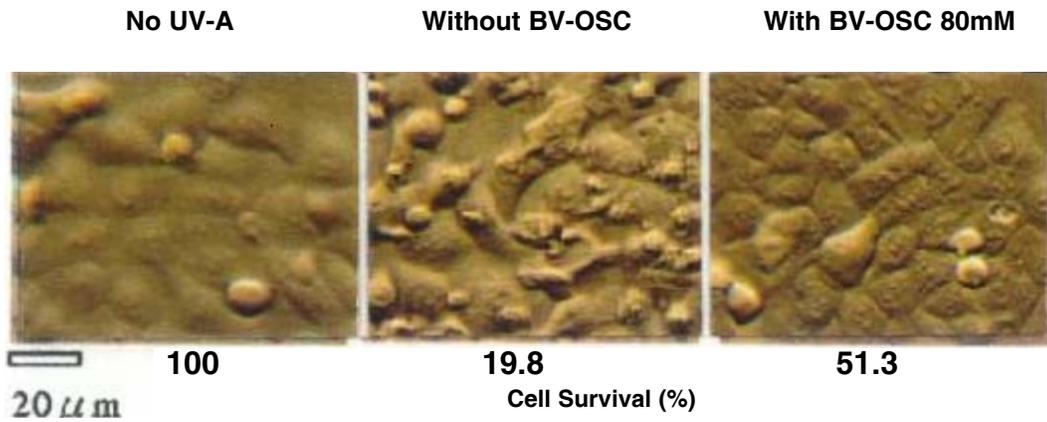
Suppression of DNA Damage Induced by UVB



DNA damage was evaluated by the comet assay. HaCaT keratinocytes which were treated with VC derivatives for 24 h, were exposed to UVB at 10 mJ/cm², n=50.

D. BV-OSC Prevents UV-A Damage

Cyto-Protective Effect of BV-OSC Against UVA Irradiation

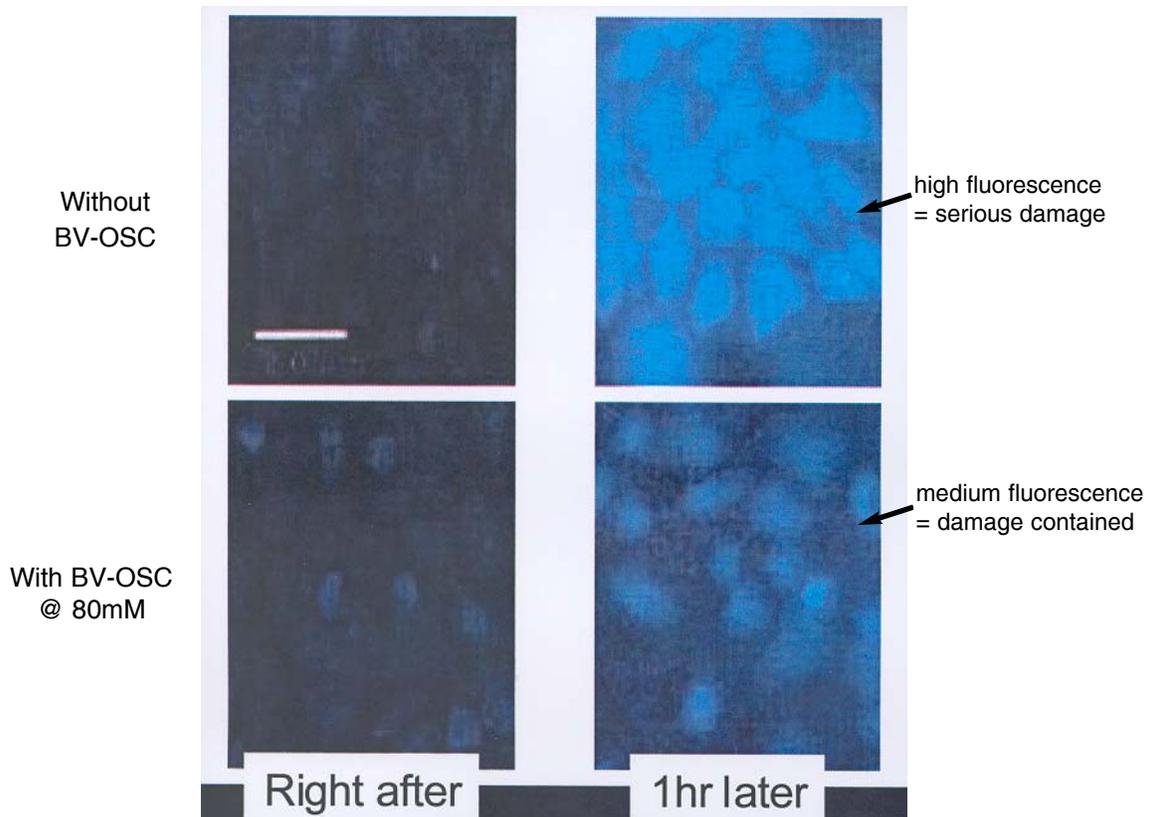


→ **Quantitative evaluation**

UVA damage can be measured by the quantity of 8-OHdG released.

**Inhibitory Effect on 8-OHdG Production
Induced by UV-A**

8-OHdG (8-hydroxy-2'-deoxyguanosine)



HaCaT cells were treated with 80 mM BV-OSC. After UVA irradiation, 8-OHdG was detected immunohistochemically using anti-8-OHdG antibody.

The application of BV-OSC inhibits the release of 8-OHdG, thereby protecting the cell against UV-A damage.

3. BV-OSC has anti-aging properties.

A. BV-OSC and Collagen Synthesis

First we observed that by adding 0.1% of BV-OSC in a fibroblast culture, the proliferation of the cells is increased by 50% (Figure 1).

Furthermore, the fibroblasts are significantly increasing collagen synthesis. It doubles with the use of 50µM of BV-OSC. The same dosage of Ascorbic Acid increases collagen synthesis by only 25% (Figure 2).

Figure 1: BV-OSC and Cell Proliferation

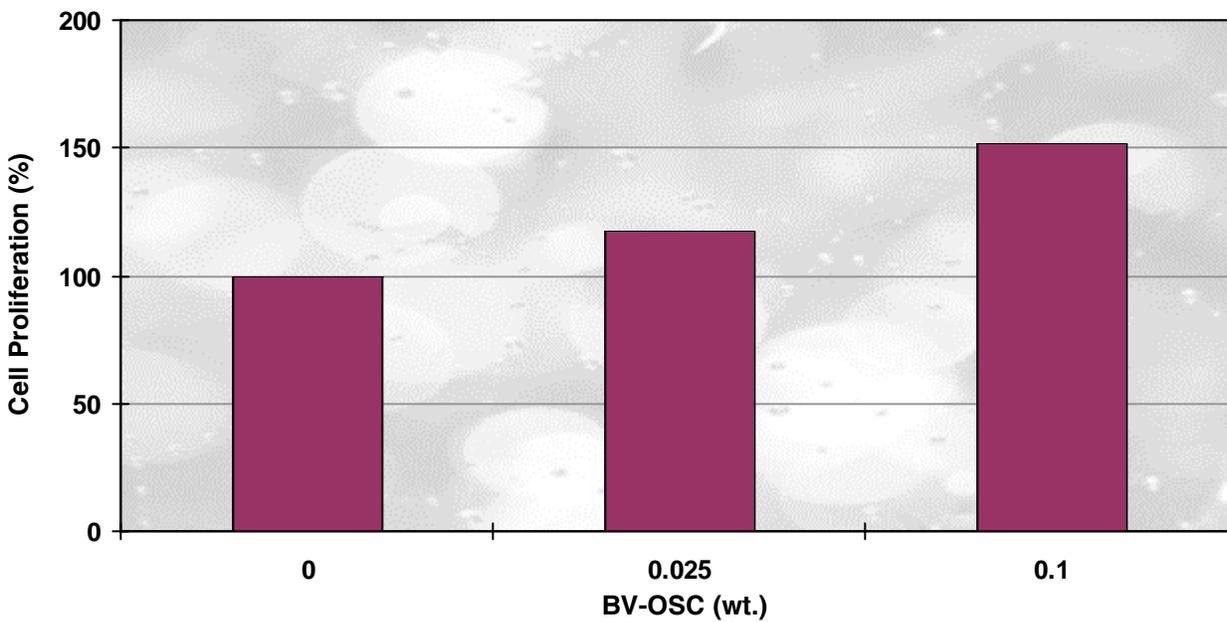
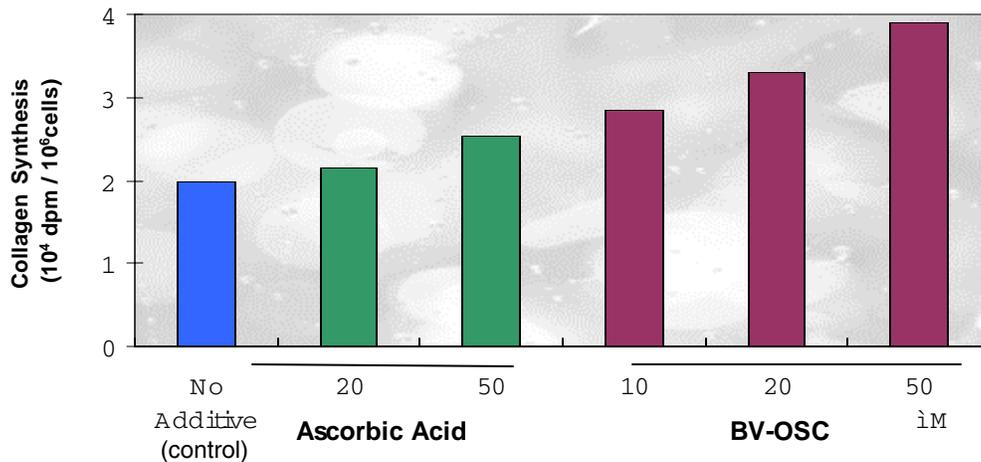
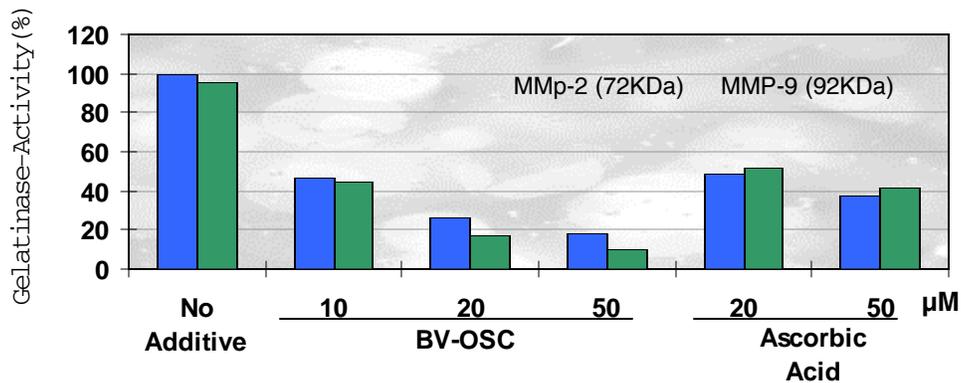


Figure 2: Comparison of Ability for Collagen Synthesis



B. BV-OSC has MMP Inhibition Effect.

Figure 3: The Ability of Inhibition of Gelatinase Activity



Measurement of MMPs:

Serum-free condition media of NHDF cells cultured for 48 hours in the presence or absence of 50 μM BV-OSC were concentrated by ultra-filtration, and were electrophoresed under non-reduced conditions on a SDS-Polyacrylamide gel containing 0.2% gelatin, followed by staining with Coomassie Brilliant Blue R250 and subsequent measurement by laser densitometry.

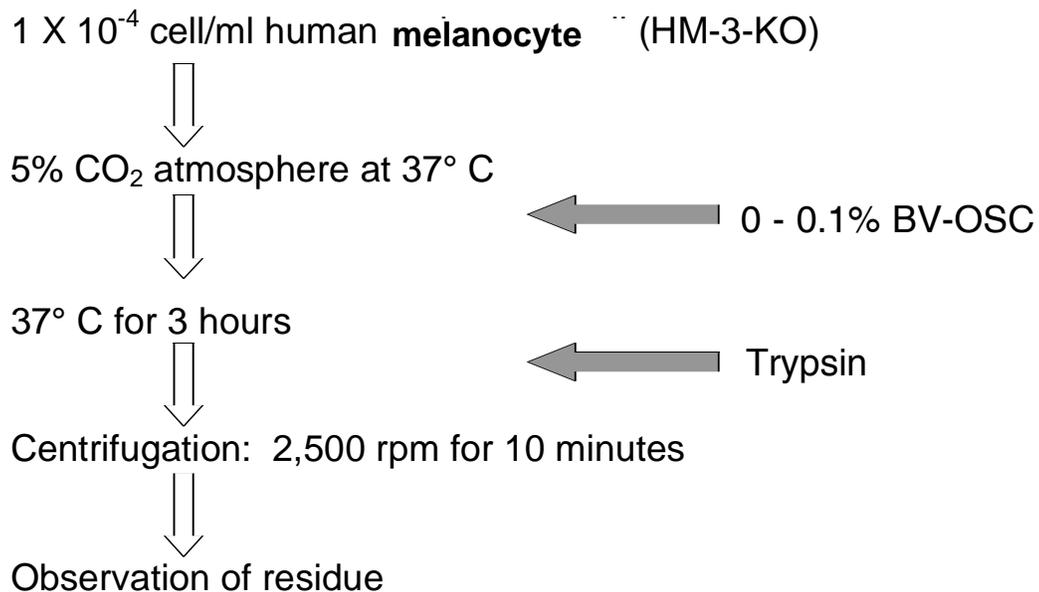
4. *BV-OSC has whitening properties.*

A. Inhibition of Melanogenesis *in vitro* test with BV-OSC

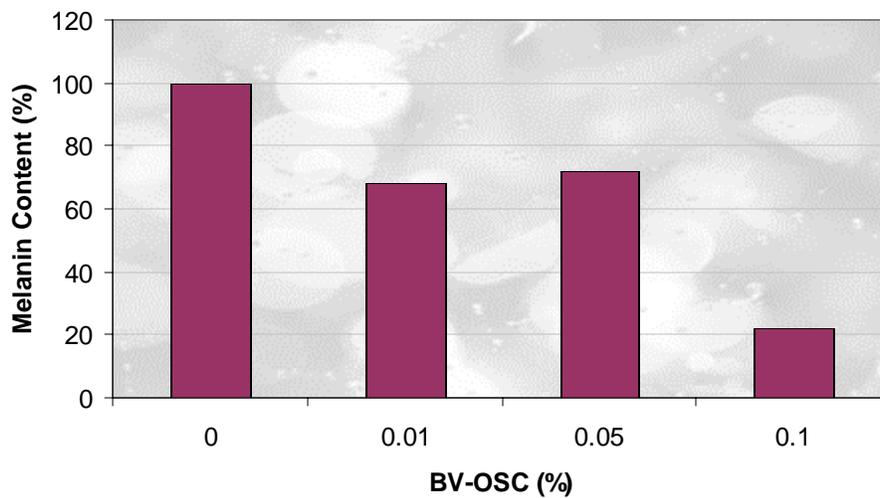
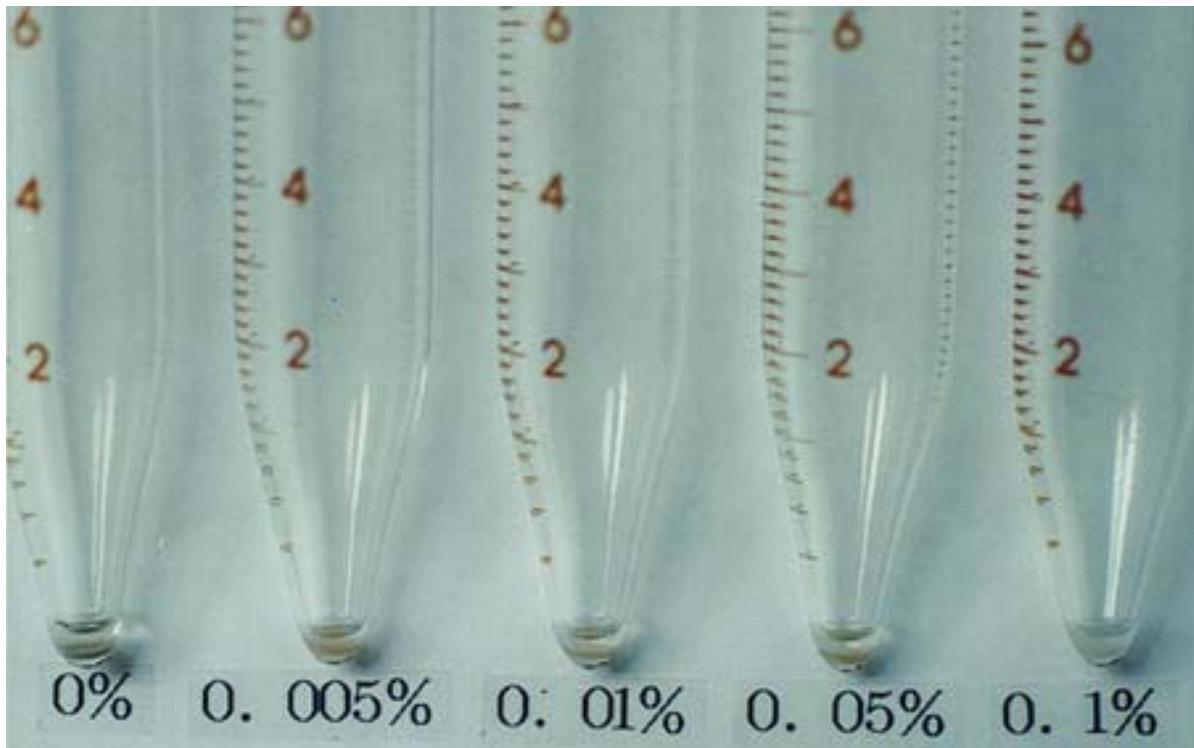
One of the many benefits of Vitamin C in cosmetic formulations is its ability to provide a more even skin tone. Occidental countries describe the activity as a "clarifying and brightening" effect, while in Asia the term "whitening" is used.

The following *in vitro* test shows that 0.1% - 0.2% of BV-OSC reduces melanogenesis by more than 80%.

Protocol for Evaluation of Inhibitory Effect of Melanogenesis



Inhibitory Effect on Melanogenesis In Cultured Human Melanocyte



Human melanocytes were treated with the medium containing BV-OSC for 4 days. After harvesting the cells, melanin contents were estimated using slot-blot method. Values were expressed as % of control.

5. BV-OSC Doctor's Application

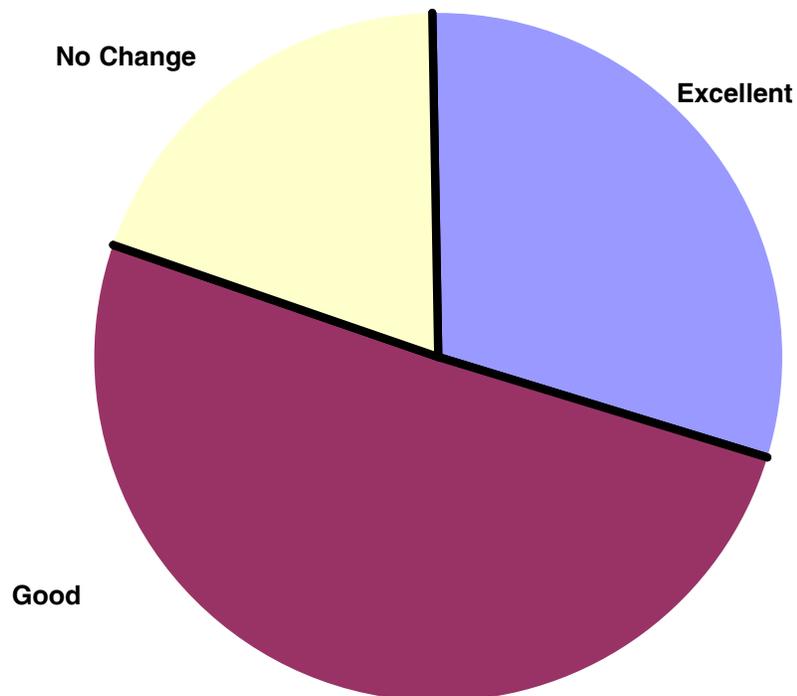
An aqueous gel with 10% BV-OSC was applied to 10 patients with acne (16-45 years old) for 2-10 months. Efficacy was evaluated according to the following scale:

- > 75% improvement: Excellent
- > 50% improvement: Good
- < 50% improvement: No Change

10% GEL FORMULATION

Water	q.s. 100%
Concentrate Glycerin	17.0%
Carbomer	0.5%
Sodium Polyacrylate	0.25%
Butylene Glycol	2.5%
BV-OSC	10.0%
Methyl paraben	0.05%
Phenoxyethanol	0.6%

TEST RESULT - ACNE TREATMENT BY 10% BV-OSC GEL



BEFORE



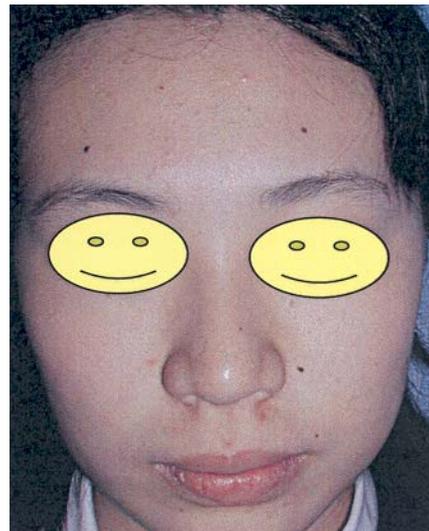
AFTER 12 WEEKS



BEFORE



AFTER 9 WEEKS



BEFORE

AFTER 16 WEEKS

