NOP Organic Acerola Ferment Procollagen Assay

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Purpose

A fibroblast cell culture model was used to assess the ability of NOP Organic Acerola Ferment to exert an effect on collagen synthesis.

Summary of Test Method

Fibroblasts are the main source of the extracelluar matrix peptides, including the structural proteins, collagen and elastin. Procollagen is a large peptide synthesized by fibroblasts in the dermal layer of the skin and is the precursor for collagen. As the peptide is processed to form a mature collagen protein, the propeptide portion is cleaved off (type I C-peptide). Both the mature collagen protein and the type I C-peptide fragment are then released into the extracellular environment. As collagen is synthesized, the type I C-peptide fragment accumulates into the tissue culture medium. Since there is a 1:1 stoichiometric ratio between the two parts of the procollagen peptide, assaying for type I C-peptide reflects the amount of collagen synthesized. Type 1 C-peptide was assayed via an ELISA based method.

Procollagen Assay

A series of type I C-peptide standards were prepared ranging from 0 ng/ml to 640 ng/ml. Next, an ELISA microplate was prepared by removing any unneeded strips from the plate frame followed by the addition of 100 µl of peroxidase-labeled anti procollagen type I-C peptide to each well used in the assay. Twenty (20) µl of either sample (collected tissue culture media) or standard were then added to appropriate wells and the microplate was covered and allowed to incubate for 3 ± 0.25 hours at 37°C. After the incubation, the wells were aspirated and washed three times with 400 µl of wash buffer. After the last wash was removed 100 µl of peroxidase substrate solution (hydrogen peroxide + tetramethylbenzidine as a chromagen) was added to each well and the plate was incubated for 15 ± 5 minutes at room temperature. After the incubation, 100 µl of stop solution (1 N sulfuric acid) was added to each well and the plate was read using a microplate reader at 450 nm.

Calculations

Procollagen Concentration

To quantify the amount procollagen present, a standard curve was generated using known concentrations of procollagen type 1-C peptide. A linear regression was then performed to establish the line that best fits these data points. Mean absorbance

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values for the test materials and untreated samples were then used to estimate the amount of procollagen type 1-C peptide present in each sample.

Results

Treatment	Mean	Stdev
1% NOP Organic Acerola Ferment	2574	137
0.5% NOP Organic Acerola Ferment	2264	243
1% Magnesium Ascorbyl Phosphate	2810	47
0.5% Magnesium Ascorbyl Phosphate	2727	222
4 mM Sodium Butyrate	1706	179
Untreated	1279	143

Table 1. Mean procollagen concentrations following treatment.

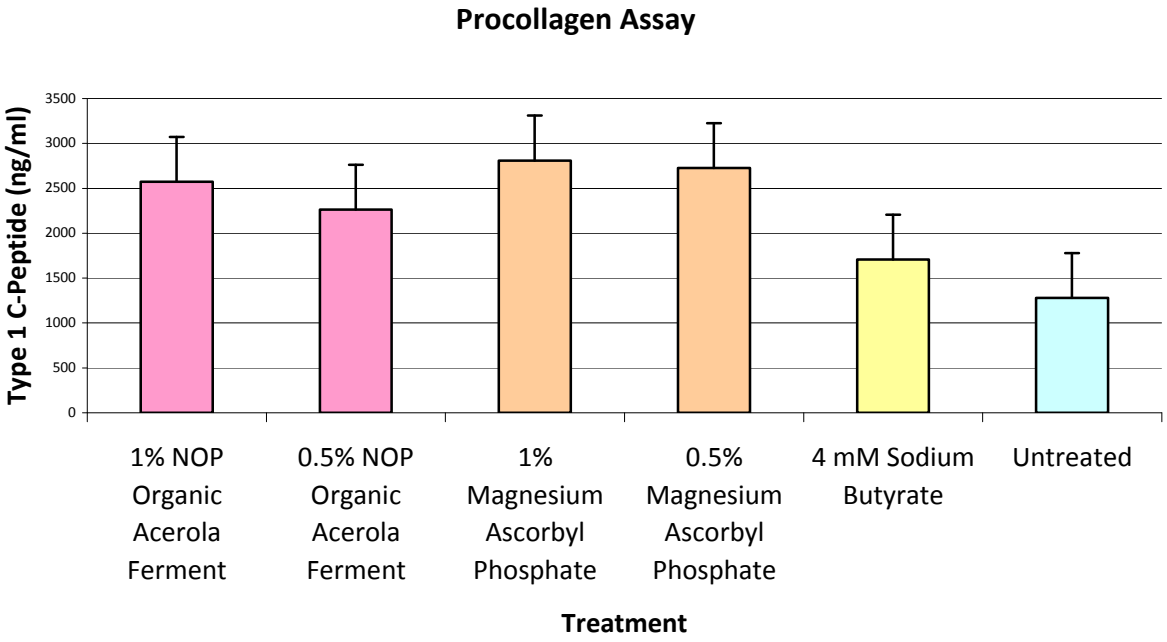

Figure 1. Comparison of the effects on procollagen levels following treatment.

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Discussion

The ELISA indicates that NOP Organic Acerola Ferment is capable of increasing the expression of procollagen type 1-C peptide in the fibroblast cell culture model. NOP Organic Acerola Ferment was shown to be comparable to Magnesium Ascorbyl Phosphate in increasing the synthesis of Collagen Type 1. These findings suggest that NOP Organic Acerola Ferment is useful in cosmetic preparations to stimulate collagen type 1 production *in situ*.