

Stimulation of Collagen Production in Human Fibroblasts

Authors: Dr Jane Tiedtke and Dr Olaf Marks, Cosmtochem International, Switzerland
Dr Jacques Morel, Cosmtochem, France

Abstract:

Collagen is produced in the fibroblasts of the human dermis and is essential for healthy, firm skin. Both the quality and quantity of collagen decrease in ageing skin, often due to the effect of external factors such as exposure to the sun, especially UVA radiation. The result of this cross-linking is that the skin loses its elasticity. In the past, topical application of soluble animal collagen was used in an attempt to stimulate the formation of collagen in the skin. However, these tests were unsuccessful, as collagen cannot penetrate the epidermis.

Vitamin C is known to stimulate collagen formation but has the disadvantage of being unstable in cosmetic formulations. This article discusses an encapsulated stabilised form of vitamin C* which not only stimulates collagen production in the human fibroblasts *in vitro* but also stimulates cell regeneration without accelerated cell death.

Introduction

Collagen plays a pivotal role in maintaining skin structure and accounts for as much as 70% of the weight of the skin. The formulation of new collagen fibres is therefore essential for healthy, firm skin¹. Type I and type III collagen are present in the highest levels in the skin, forming 80% and 15% of the total collagen present respectively¹. Type IV collagen is the main component of the lamina densa, a 50nm thick layer in the basement membrane zone¹⁸ and type VII collagen is a major constituent of the anchoring fibrils beneath the lamina densa, at the dermal-epidermal interface¹⁸. Type V collagen is found pericellularly. During ageing, fibroblasts in the skin do not produce so much collagen. It has been generally believed that type III collagen is found in much higher levels in young skin but decreases significantly with age²⁰. Its role has been correlated with tissue extensibility^{5,21}, being replaced in the first instance by type I collagen which forms a more rigid structure. However there has been more recent research to suggest that this

relationship is not so clear cut⁵. During ageing the levels of collagen not only decrease¹⁶ but the collagen fibres begin to cross-link^{14,15} and this is often due to the effect of external factors such as exposure to the sun, especially UVA radiation⁶. The result of this cross-linking is that the skin loses its elasticity. In ageing skin a higher proportion of cross-linked insoluble collagen is found, while younger, healthy, taut skin possesses a higher level of fresh-formed, soluble collagen. In the past, topical application of soluble animal collagen was used in an attempt to stimulate the formation of collagen in the skin. However these tests were unsuccessful as collagen cannot penetrate the epidermis.

Collagen is formed in the skin by certain fibroblast cells. It is possible to stimulate the proliferation of these fibroblasts and to induce them over a limited time to produce more collagen. This procedure is usually not feasible over a long period because the proliferation of fibroblast cells is closely linked with accelerated cell death.

This article discusses an encapsulated active* which not only stimulates collagen production in the human fibroblasts *in vitro*, but also stimulates cell regeneration without accelerated cell death.

Test Product description

The test product is a nano-encapsulated, stabilized, vitamin C-derivative* (magnesium-L-ascorbyl phosphate) in the form of a so-called "intracellular-booster", which is a serum containing a high concentration of clearly defined carriers or delivery capsules. The nano-capsules have an average particle size of 150-200 nm, enabling them to reach the appropriate layers of the dermis, which allows them to interact in the process of collagen synthesis. As a strong reducing agent it acts on the redox-systems for the hydroxylation of proline to hydroxyproline, a characteristic part of the collagen structure.



Natural Ingredients

During product development particular attention was paid to the following parameters of the encapsulation system used for the encapsulated vitamin C derivative* :

- stability
- size
- active surface area
- concentration of carriers

Stability of the encapsulation system

The nano-capsules are very stable, even under extreme conditions including ultra-centrifugation for 5 minutes at 20,000g. They are stable in most product types, except oils and those containing high levels of surfactants such as bath and shower products, liquid soaps and hair shampoos. The stability and the presence of the encapsulated vitamin C derivative* in the finished preparation can be checked by measuring the Zeta-potential.

Efficacy of an encapsulated vitamin C derivative* as a collagen stimulator

Methods

MTT cell stimulation assay

Measurement of cell proliferation was made using the MTT method²⁵. MTT is now a widely accepted method which uses the reduction of tetrazolium salts in the evaluation of both cell proliferation and cell death²⁶. The yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, partly by the action of dehydrogenase enzymes to generate reducing equivalents of NADH and NADHP. The resulting intracellular formazan can be solubilized and quantified by spectroscopic means. After elimination of the culture medium and dissolution of the crystals by DMSO (dimethyl sulphoxide), the optical density was read at 570 nm; this reading being directly proportional to the number of live cells present.

Collagen stimulation

An *in vitro* cell culture test was carried out to demonstrate the activity of the encapsulated vitamin C derivative* on the growth of human fibroblasts. Cells were cultivated in 10% foetal calf serum. Test cultures were prepared with a solution of unencapsulated and

encapsulated* vitamin C derivatives and benchmark or control cultures were prepared with empty liposomes.

Collagen synthesis was measured in a culture of human fibroblasts by an immuno-fluorescence technique using monoclonal anti-collagen antibodies. The anti-collagen antibodies were obtained by the reaction of mice splenocytes immunized with human collagen cells. An anti-collagen antibody is tagged with a fluorescent marker and is used to locate the sites of collagen synthesis. The test results were evaluated by comparing the reference without active matter with the fluorescence in the cells treated with the encapsulated vitamin C derivative* and by measuring the intensity of fluorescence.



Fig. 1 Control : Culture of human fibroblasts developed with monoclonal fluorescent anti-collagen antibodies

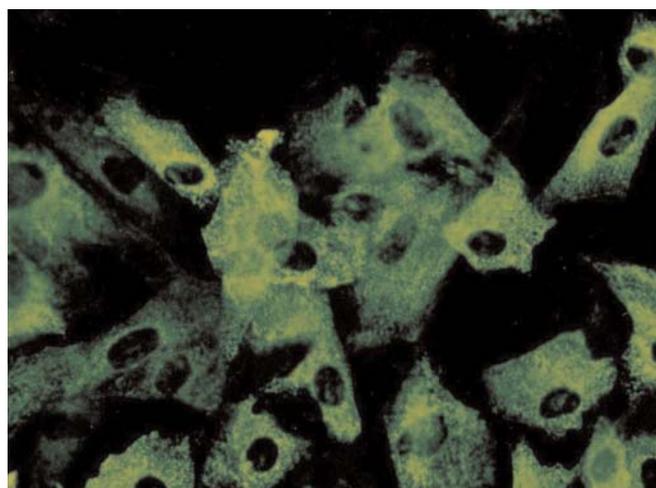


Fig. 2 Test : Culture of human fibroblasts treated with 4% of the encapsulated vitamin C derivative*, developed with monoclonal fluorescent anti-collagen antibodies

Results

Cell stimulation

The results of the MTT test confirmed that the test product was not cytotoxic and stimulated cell growth compared to the control.

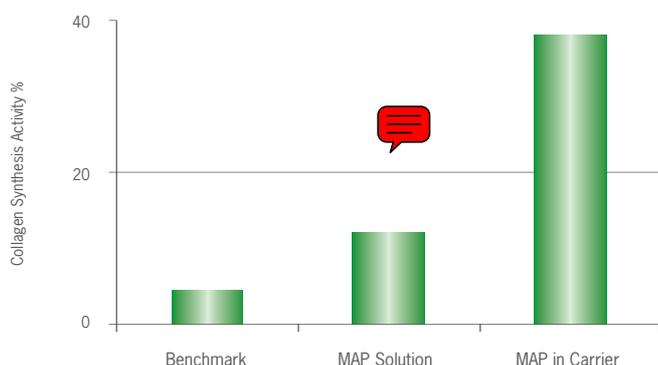


Fig. 3. : An encapsulated vitamin C derivative* : Cell culture tests on human fibroblasts.

Collagen stimulation

Cells treated with the encapsulated vitamin C derivative* showed an increase in fluorescence and thus collagen production (Fig. 2) compared to the control (Fig. 1).

The fluorescence method shows a multiplication by factor 24 of the optical density of the cell culture caused by collagen synthesis of a culture of 10^5 cells/ml in 48 hours.

The results of the fluorescent measurement are shown in graphic form in Fig 3. Magnesium ascorbyl phosphate (MAP) in its non-encapsulated form in solution, stimulates the synthesis of Collagen 2.8 times relative to the benchmark or control with empty liposomes. Magnesium ascorbyl phosphate in its encapsulated form* stimulates the synthesis of collagen 25.8 times relative to the benchmark.

The results of the fluorescence measurement gave a value of about 63 ng collagens for the reference culture and 1500 ng collagens for the culture stimulated by 4% of the encapsulated vitamin C derivative*.

Safety

- The test product* has been shown not to be cytotoxic
- Additional toxicological tests showed the test product* to be non-irritating to eye and skin
- A CIR report⁸ concluded that “the clinical experience in which ascorbic acid was used on damaged skin with no adverse effects and the repeat insult patch test (RIPT) using 5% ascorbic acid with negative results supports the fact that this group of ingredients (including magnesium ascorbyl phosphate) does not present the risk of skin sensitization”.

Discussion

Vitamin C has been documented in the literature as having antioxidant/free radical scavenging^{8,10,17,19}, collagen stimulating^{8,13,17,22} and skin whitening¹⁷ properties. However the incorporation of vitamin C into cosmetic products is problematic because it is not very stable^{11,13}. Magnesium ascorbyl phosphate is a vitamin C derivative which has much better stability^{11,13} and this has also reported to have skin lightening^{9,24}, collagen stimulating¹³ and antioxidant properties^{12,23}; however in the unencapsulated form it is poorly absorbed by the skin from a topical application¹⁰. To address the problems of stability exhibited by vitamin C and poor penetration by MAP, a stabilized form of vitamin C, an encapsulated form of MAP, was developed and tested against the unencapsulated form for the ability to stimulate collagen formation.

Conclusion

The results show that the nano-encapsulation of magnesium ascorbyl phosphate (MAP)* boosts both new cell growth and the ability of MAP to stimulate collagen synthesis in fibroblasts.

* the encapsulated vitamin C derivative used in this study is sold under the Cosmetochem tradename Collagen Stimulation Factor MAP

References

1. Oikarinen, A. (2006) Connective tissue and aging. Int. J. Cos.Sci. 26 : 107.

2. Cook, T.H. (1989) Mechanical properties of human skin with aging. In: Balin, A.K. & Kligman, A.M. eds. pub. Raven Press, New York, pp. 205-226.
3. Kligman, L.H. (1989) Skin changes in photo-ageing: Characteristics, prevention and repair. In: Balin, A. K. & Kligman, A.M. eds.pub. Raven Press, New York, pp. 331-346.
4. Miller, E.J. (1984) Chemistry of collagens and their distribution. In : Piez, K.A. Raddi, A.H. eds. Extracellular matrix. Elsevier Science, New York, pp. 41-81.
5. Mays, P.K., Bishop, J.E. & Laurent, G.J. (1988) Age-related changes in the proportion of types I and III collagen. Mech. Ageing Dev. 45 (3): 203-12.
6. Rittié, L. & Fisher, G.J. (2002) UV light-induced signal cascades and skin aging. Ageing Research Reviews 1 : 705-720.
7. Chanut-Delalande, H. et al. (2004) Development of a functional skin matrix requires deposition of collagen V heterotrimers. Molecular and Cellular Biology 24 (13) : 6049-57.
8. Elsmore, A.R. (2005) Final report on the safety assessment of L-ascorbic acid, calcium ascorbate, magnesium ascorbyl phosphate, sodium ascorbate and sodium ascorbyl phosphate. Int. J. Toxicol. 24 (2) : 51-111.
9. Parvez, S. et al. (2006) Survey and mechanism of skin depigmenting and lightening agents. Phytother. Res. July 14th (epub. ahead of print).
10. Pinnell, S.R. et al. (2001) Topical L-ascorbic acid : percutaneous absorption studies. Dermatol. Surg. 27 (82) : 137-42.
11. Austria, R., Semenzato, A. & Bettero, A. (1997) Stability of vitamin C derivatives in solution and topical formulations. J. Pharm. Biomed. Anal. 15 (6): 795-801.
12. Kobayashi, S. et al. (1996) Protective effect of magnesium-L-ascorbyl-2 phosphate against skin damage induced by UVB irradiation. Photochem. Photobiol. 64 (1): 224-8.
13. Geesin, J.C., Gordon, J.S. & Berg, R.A. (1993) Regulation of collagen synthesis in human dermal fibroblasts by the sodium and magnesium salts of ascorbyl-2-phosphate. Skin Pharmacol. 6 (1): 65-71.
14. Robert, L. (2006) An original approach to ageing: an appreciation of Fritz Verzar's contribution in the light of the last 50 years of gerontological facts and thinking. Gerontology 52 (5) : 268-74.
15. Avery, N.C. & Bailey, A. J. (2006) The effects of the Maillard reaction on the physical properties and cell interactions of collagen. Pathol. Biol. (Paris) Sept. 6 (epub. ahead of print).
16. Talwar, H.S. et al. (1995) Reduced type I and type III procollagens in photodamaged adult human skin. J. Invest. Dermatol. 105: 285-290.
17. Shih, Y. & Zen, J-M. (1999) Determination of magnesium ascorbyl phosphate in cosmetic bleaching products using a disposable screen-printed carbon electrode J. Chin. Chem. Soc.46 (6): 865-70.
18. Hata T. et al. (2006) Age induced duplication of epidermal lamina densa a characteristic structure of the basement membrane IFSCC Congress, Osaka, October 16th-19th, 2006.
19. Mizutani, Y. et al. (2006) A novel anti-aging strategy through the use of lipid peroxide. IFSCC Congress, Osaka, October 16th-19th, 2006.
20. Epstein, E. H. (1974) Alpha I (III), Human skin collagen: Release by pepsin digestion and preponderance in foetal life. J. Biol. Chem. 249: 3225-31.
21. Burgeson, R.E. Collagens of the skin (1987) Curr. Probl. Dermatol. 17 : 61-75.
22. Tajima, S. & Pinell, S.R. (1996) Ascorbic acid preferentially enhances type I and III collagen gene transcription in human skin fibroblasts. J. Dermatol.Sci. 11 : 250-53.
23. Kobayashi, S.M. et al. (1998) Post administration protective effect of magnesium-L- ascorbyl phosphate on the development of UVB-induced cutaneous damage in mice. Photochem. Photobiol. 67 : 669-75.
24. Kameyama, K.C. et al. (1996) Inhibitory effect of magnesium-L-ascorbyl-2- phosphate (VC-PMG) on melanogenesis *in vitro* and *in vivo* J. Am. Acad. Dermatol. 34 : 29-33.
25. trevigen.com
26. Mossmann, T. (1983). Rapid colorimetric assay for cellular growth and survival : application to proliferation and cytotoxicity assays. Immunol. Methods 65. 55-63.

Author's Biographies

Dr Jane Tiedtke

BSc and PhD in Microbiology. Spent 15 years with Rohm and Haas Company in France in both marketing and technical posts in their Consumer and Industrial Specialities Division. Joined Cosmtochem International Ltd. based in Switzerland in 2001 and currently holds the position of Head of Marketing.

Dr Jacques Morel

Diploma in Biochemistry from the University of Nice. Worked for French perfumery industry before becoming General Manager of Cosmtochem France in 1988 until he retired in 2006.

Dr Olaf Marks

Ph.D (Dr. Phil) in chemistry at the University of Zurich. After several years of scientific research at the university joined Cosmtochem as Head of Marketing in 1986. Currently holds position of CEO and member of the board at Cosmtochem International Ltd.