Clinical and Histological Efficacy of a Secretion of the Mollusk *Cryptomphalus aspersa* in the Treatment of Cutaneous Photoaging

María José Tribó-Boixareu, MD; Concepción Parrado-Romero, MD; Badr Rais, PhD; Eduardo Reyes, PhD; María Alejandra Vitale-Villarejo, MD; Salvador González, MD

In this article, the authors describe the efficacy of a natural secretion of *Cryptomphalus aspersa* (SCA) in cutaneous photoaging. This placebo-free, open-label study comprised 15 women exhibiting facial photoaging. Treatment with SCA for 3 months induced a significant reduction in photoaging on the Glogau scale. Silicone replica analysis showed significant improvements in skin roughness and wrinkle depth. Histopathological analysis revealed a marked improvement in both dermal and epidermal architecture, as well as epidermal proliferation and the percentage of cutaneous area covered by microvessels. In summary, treatment with SCA for 3 months resulted in a significant improvement of the clinical and histological signs of photoaging, and these correlated with both subjective and objective assessments of chronic photodamage.

Dr. Tribó-Boixareu is Faculty, Dermatology Department, Hospital del Mar, Barcelona, Spain. Dr. Parrado-Romero is Associate Professor, Department of Morphological Sciences, Faculty of Medicine, Malaga University, Spain. Dr. Rais is Employee, Biochemistry and Cell Biology Department, Bio European Skin Testing, Barcelona, Spain. Dr. Reyes is Employee, Scientific Department, Industrial Farmaceutica Cantabria SA, Madrid, Spain. Dr. González is Faculty, Dermatology Services, Memorial Sloan-Kettering Cancer Center, New York, New York.

Drs. Reyes and Vitale-Villarejo are employees of and Dr. González is a consultant for Industrial Farmaceutica Cantabria SA.

Correspondence: Salvador González, Faculty of Dermatology Service, Memorial Sloan-Kettering Cancer Center, 160 E 53rd St, New York, NY, 10022 (gonzals6@mskcc.org).

Skin aging due to the passage of time is modulated by the interaction between individual genetics and environmental factors. Genetic determinants and environmental factors, particularly UV radiation, may induce premature skin aging or photoaging.[1] Photoaging causes functional, anatomical, and structural modifications in the exposed areas,[2-6] and UVB radiation produces direct damage to skin cells' DNA and also modulates the activity of cytokines and adhesion molecules.[7-8] On the other hand, UVA radiation induces the formation of reactive oxygen species, which also damages nuclear and mitochondrial DNA and activates matrix metalloproteinases.[1,5-9]

The signs of photoaging include epidermal thickening, keratinocyte atypia, loss of polarity and increased melanogenesis.[10] The dermal fibrillar network is disorganized.[11]

Other changes include collagen reduction and the appearance of fragmented collagen fibrils; presence of senescent fibroblasts[12,13]; loss of function of glycosaminoglycans (hyaluronic acid and dermatan sulphate); and alterations in the cutaneous microvasculature.[14,15] All these findings result in characteristic clinical features, such as dryness, roughness and sallowness, deep wrinkles, and lack of firmness and elasticity.[1,2,16,17]

Concern regarding the visible manifestations of skin aging represents one of the leading reasons for dermatological consultation. Topical therapy is the most widely accepted treatment by the majority of patients.[18-21] In this article, the authors provide preliminary evidence of the effect of a novel natural product derived from the mollusk *Cryptomphalus aspersa*[22] (SCA), which is a glycosaminoglycan-enriched substance that induces skin regeneration.[23,24] Based on this evidence, the authors hypothesized that SCA can be useful to reverse the effects of photoaging. In this study, the authors investigate the effect of SCA treatment in a small group of women (n=15) showing cutaneous photoaging and found that treatment with SCA for 3 months resulted in a significant improvement of the clinical and histological features of photoaging, thus postulating its efficacy as a potential antiaging compound for the skin.

MATERIALS AND METHODS

SCA Preparation

Preparation for SCA was executed according to US patent 5538740 and has been described in detail elsewhere.[22]

Study Design

Fifteen volunteers were included in this nonrandomized, open-label study. Participants were informed of the requirements of the study including 2 biopsy collections, and they signed a written informed consent under an institutional review board–approved study protocol. The study group comprised 15 women aged 35 to 65 years (mean [SD] age=52.1 [6.2]) with Fitzpatrick skin types II (53.8%) and III (46.2%), and features consistent with facial photoaging. Prior to the beginning of the treatment, each participant underwent a 1-month washout period, during which all topical antiaging treatments were discontinued. Participants were instructed to apply an SCA 8% emulsion in the morning and a highly concentrated SCA 40% liquid formulation at night on a daily basis for a period of 3 months. Assessments were performed at the initial consultation at baseline and days 30 and 90.

Clinical Efficacy Variables

The researcher recorded the following clinical variables: presence of fine lines and wrinkles, sallowness, telangiectasias, reduced cutaneous elasticity, roughness, irregular mottled pigmentation, rhytides, and solar lentigines. Other parameters included sebaceous hyperplasia, actinic keratosis, and presence of malignant neoplastic processes.

PHOTODAMAGE

A severity score between 1 and 6, with 6 being the most severe, was assessed using the Glogau scale.[16,25] One of the authors, Dr. María José Tribó-Boixareu, evaluated treatments with each individual participant during each clinical visit and scored treatments from 0 (unchanged) to 6 (marked improvement).

Clinical Assessment of the Effect of SCA

The clinical efficacy of SCA was measured by confocal profilometry of silicone replicas. The technique has been described in detail elsewhere.[26,27] Briefly, 1-cm-wide silicone impressions were taken from the periocular area at baseline and day 90. The following parameters were assessed: average roughness of the profile; root-mean-square roughness of the profile; maximum height of the roughness profile; maximum peak height of the roughness profile; maximum valley depth of the roughness profile; and distance between the highest peak and the deepest valley of the assessed profile.

Histology

Histological analysis was performed in 3-mm diameter samples collected from the periocular areas of all the patients. Biopsies were obtained at baseline and day 90. Samples were stained with hematoxylin and eosin to visualize the architecture and determine epidermal thickness. Unna stains were used for visualization of the elastic fibers and assessment of cutaneous elastosis.[28]

Immunohistochemistry

The sections used for immunohistochemical analysis were deparaffinized and incubated overnight at 4ºC with anti–Ki-67-*mab* for proliferating keratinocytes, or anti–CD31-*mab* for endothelial cells, and appropriate secondaries. Each section was counterstained with hematoxylin and eosin and dehydrated.

Quantitative Histologic Analysis

Immunohistochemistry images were quantified using an image processing program, and the percentage of proliferating epidermal cells was calculated and expressed as epidermal proliferating index.[29] For microvessel quantification and area, microvascular density was measured in 5 to 6 spots, and $CD31^+$ cell (endothelial cell) clusters were included in the microvessel count.

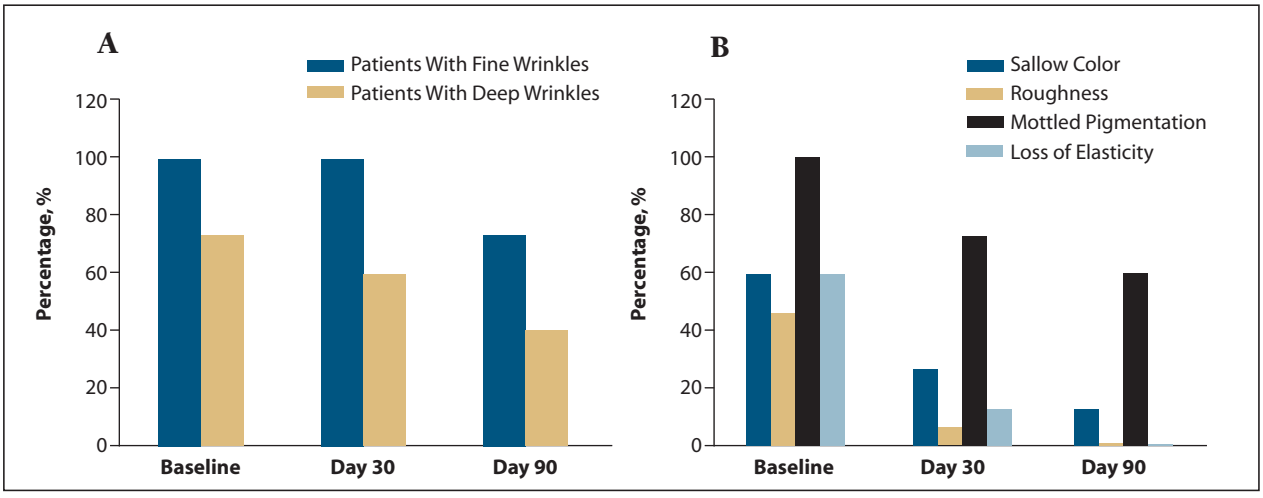

Figure 1. Effect of *Cryptomphalus aspersa* on the presence of fine and deep wrinkles ($P<.05$ and $P<.025$, fine and deep wrinkles, respectively) (A), and the effect of *Cryptomphalus aspersa* on different features of photoaging (B). Percentages are shown at the initiation of the treatment at baseline and days 30 and 90.

Statistical Analysis

The Cochran test was used to assess potentially significant differences in the clinical evolution between the 3 measurements at baseline and days 30 and 90, as well as the individual and global assessment by the researcher and the participants. The Wilcoxon rank sum test was used to assess differences in skin photoaging severity and histological parameters between the initiation of the study and its completion at 3 months. This test was also used to characterize the individual and global assessment of the researcher and the patients between visits. The normal distribution of the analysis of silicone replicas by profilometry was demonstrated via unidirectional analysis of the sample using statistical software. In all cases, standardized kurtosis coefficient was within the expected range for normal distribution data (−2, +2); thus, parametric statistical criteria were applied.

RESULTS

Clinical Efficacy of SCA

We first established the severity of cutaneous photoaging before and after treatment with SCA using the Glogau scale. This scale showed a significant reduction ($P<.0001$) of photoaging signs after treatment with SCA at day 90 as compared with the same participants at the beginning of the study. The percentage of participants showing fine wrinkles and deep wrinkles decreased gradually and significantly ($P<.05$) during the treatment. The authors observed a 45.5% decrease in participants showing deep wrinkles after the treatment (11/15 at baseline vs 6/15 at day 90, $P=.025$) (Figure 1A). The sallow appearance of skin showed a 50% reduction at day 30 ($P<.02$) and 77.8% at day 90 ($P<.008$). Loss of cutaneous elasticity also improved very significantly during the treatment (9/15 at baseline; 2/15 at day 30, $P<.008$ vs baseline; 0/15 at day 90, $P<.003$ vs baseline). Dryness and cutaneous roughness disappeared in 86% of patients at day 30 ($P<.01$) and in 100% of them at the end of the treatment at day 90 ($P<.008$) (Figure 1B). Finally, irregular pigmentation, which constitutes a frequent sign of photoaged skin, exhibited a 40% decrease at the end of the treatment ($P<.01$). There was no significant decrease of ephelis or lentigines, and no malignant neoplasms were reported during the assay.

GLOBAL PATIENT ASSESSMENT

A majority of the participants involved in the study reported a moderate to significant improvement (score=2/3) for the individual parameters included in the questionnaire at day 30, which evolved into marked improvement (score=3/3) by the end of the study at day 90. The global assessment score was 11/15 at day 30, increasing to 13/15 at day 90. Analysis revealed a statistically significant difference ($P<.05$) between the total score at days 30 and 90 (Table).

GLOBAL RESEARCHER ASSESSMENT

The researcher's perception scores were very similar to those provided by the participants. Assessment of the global score was 11/15 at day 30, improving to 14/15 at day 90, indicating a significant global improvement induced by treatment with SCA. Improvement at day 90 was observed in most of the assessed parameters, especially hydration ($P<.003$) (Table).

Overall, the tolerance and cosmetic acceptance of the treatment with SCA ranged from very good to good. Only

Significance of the Global Assessment of the Researcher and the Participants on Day 90 Versus Day 30

	Smoothness, *P* Value	Hydration, *P* Value	Total Score, *P* Value
Participants' Assessment	.08	.08	.04
Researcher's Assessment	.41	.003	.002

one patient experienced an adverse effect (folliculitis) at day 30, apparently unrelated to the treatment with SCA.

In summary, the data reveal a significant correlation between the global opinion of the patient and the researcher (Spearman rank correlation >0.85; $P<.0001$).

Silicon Replica Assessment

The mean values for roughness and wrinkle depth were obtained before the initiation of the treatment at baseline and after SCA treatment at day 90 by profilometry. The authors measured the mean distance between the highest peak and the deepest valley of all the profiles assessed for each patient before and after treatment, as well as the differences observed in roughness parameters. These parameters revealed an average 13% improvement in wrinkle depth, but as high as 30% in several participants (Figure 2). A nonparametric statistical test indicated that the improvement was statistically significant ($P=.021$). Also, an 18% improvement in cutaneous microroughness was observed, reaching 30% in some participants ($P=.008$).

Histopathological Analysis

Staining of biopsies of periocular tissue collected at baseline and day 90 showed a marked improvement in the architecture of the dermis and epidermis at the end of treatment (Figure 3). The authors also found a significant reduction in mean [SD] epidermal thickness (64 [4] vs 55 [4]; $P=.025$) (Figure 3), along with significant increases of the mean [SD] epidermal proliferation index (11 [1] vs 14 [2]; $P<.04$). On the other hand, there was an apparent reduction of elastosis at the papillary dermis and increases of the area percentage occupied by microvessels ($P<.002$). Finally, the authors also noticed an almost significant increase ($P<.075$) in the number of mean [SD] blood vessels per mm^2 (50 [10] vs 80 [8]).

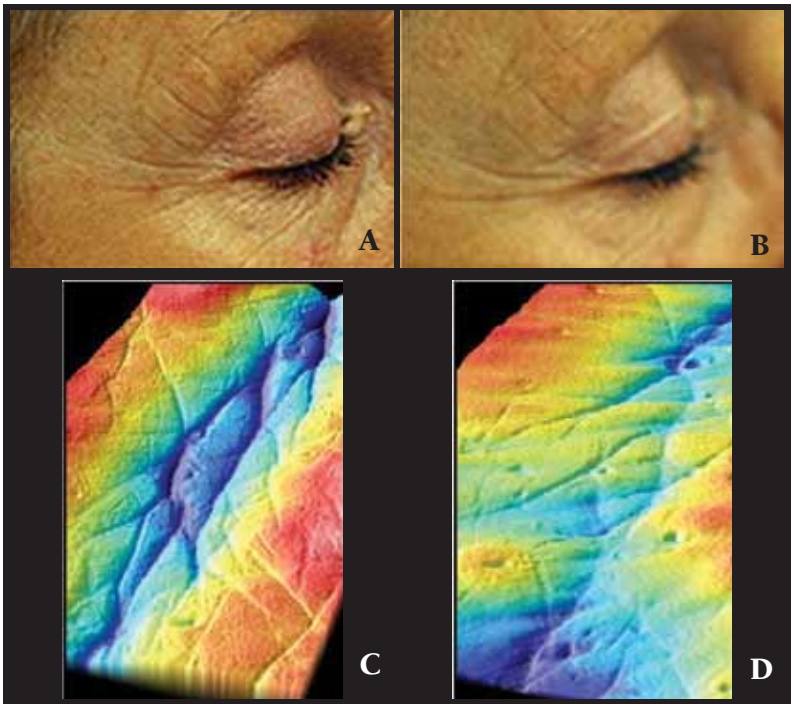

Figure 2. Effect of *Cryptomphalus aspersa* treatment on wrinkle depth assessed by profilometry. Patient before (A) and 90 days after treatment with *Cryptomphalus aspersa* (B). Corresponding 3-dimensional profilometry photographs, with grading from red (more superficial) to blue (deeper) is used to enhance visualization of the depth of the wrinkles (C, D).

DISCUSSION

In this report, the authors have shown preliminary evidence that topical treatment with SCA may reverse some of the features of photoaging. This compound had been previously shown to induce skin regeneration in cases of radiodermatitis.[23]

Clinical assessment of the effect of SCA using an arbitrary scale for cutaneous photoaging[16] established the efficacy of SCA in reversing some of the features of photoaging. In addition, profilometry analysis of selected areas of the skin of the patients revealed a positive effect of

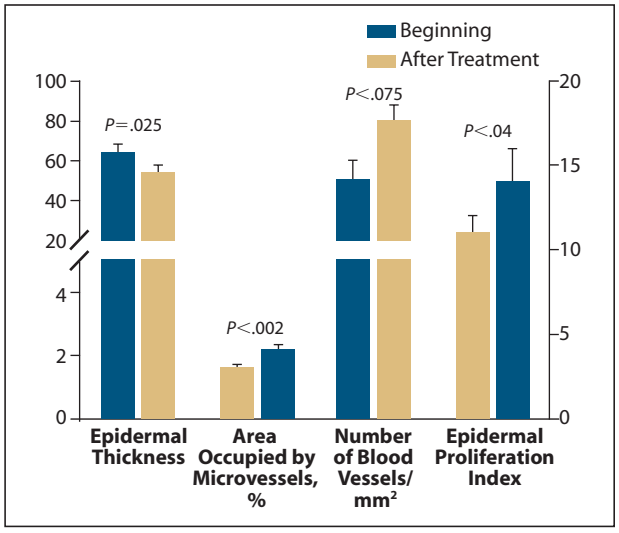

Figure 3. Evaluation of the effect of *Cryptomphalus aspersa* on epidermal thickness, area occupied by microvessels, number of blood vessels/mm^2, and epidermal proliferation index. Biopsies were collected at baseline and day 90. Note the improvement on the appearance of the outer layer of the epidermis, as well as the delineation of the dermal boundary. A marked reduction in epidermal thickness and increase of the area occupied by microvessels, number of blood vessels, and epidermal proliferation index was found after *Cryptomphalus aspersa* treatment.

SCA in the overall roughness of the skin and particularly in wrinkle depth. This is likely due to its antioxidant and matrix-remodelling effect, but contributions from the vehicle components of the secretion cannot be ruled out. The observed improvement in roughness is likely related to improved hydration due to a number of factors, such as replenishment of the skin cellularity and rearrangement of the fibrillar components of the skin. These effects were confirmed using conventional histological techniques.

We also observed that continuous use of SCA increased skin thickness and decreased the signs of elastosis, suggesting enhanced skin cell proliferation and rearrangements of the fibrillar components of dermis and epidermis. The beneficial effect of SCA can be direct via its effect on skin cell proliferation and matrix remodeling, or indirect by enhancing vascularization of the skin. Proper blood traffic provide the neighboring tissue with the nutrients and oxygen required for proliferation, thus enhanced irrigation is essential for a rejuvenation effect on photodamaged skin.

Other mechanisms to explain the effect of SCA may include a modest inhibition of metalloproteinases expression, activation, or both, which would result in decreased overall degradation of the connective tissue of the skin.[24] The precise mechanism by which SCA induces matrix remodelling is not clear. It can be related to enhanced deposition of extracellular matrix proteins, which would account for thicker lattices; enhanced expression or activation of integrin receptors, as it has been shown for other natural compounds, such as ginseng[30]; or even activation of contractility pathways such as myosin II, which results in enhanced collagen reorganization.[31] In summary, this study provides preliminary evidence of the potential usefulness of SCA as a photoaging palliative remedy. However, in light of the limitations of the present study, further research including large comparative and placebo-containing, double-blind, randomized studies is required to establish the usefulness of this natural compound in the treatment of photoaging.

***Acknowledgment*—**This study was supported by a research grant from Industrial Farmaceutica Cantabria SA, Madrid, Spain, of which Salvador González, MD, has a consultant role. The authors also thank Dr. Miguel Vicente-Manzanares, MD, Francisca Rius-Diaz, PhD, and Carmen Rios-Barranquero, RN.

REFERENCES

1. Fisher GJ. The pathophysiology of photoaging of the skin. *Cutis*. 2005;75(suppl 2):5-8; discussion 8-9.
2. Petersen MJ. Aging of the skin. In: Freinkel RK, Woodley DT, eds. *The Biology of the Skin*. New York, NY: The Parthenon Publishing Group; 2001:209-217.
3. Piérard GE. Ageing across the life span: time to think again. *J Cosmet Dermatol*. 2004;3:50-53.
4. Piérard GE, Uhoda I, Piérard-Franchimont C. From skin microrelief to wrinkles. an area ripe for investigation. *J Cosmet Dermatol*. 2003;2:21-28.
5. Beissert S, Granstein RD. UV-induced cutaneous photobiology. *Crit Rev Biochem Mol Biol*. 1996;31:381-404.
6. Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. *J Photochem Photobiol B*. 1992;14:105-124.
7. Krutmann J, Grewe M. Involvement of cytokines, DNA damage, and reactive oxygen intermediates in ultraviolet radiation-induced modulation of intercellular adhesion molecule-1 expression. *J Invest Dermatol*. 1995;105(suppl 1):67S-70S.
8. Wang XY, Bi ZG. UVB-irradiated human keratinocytes and interleukin-1 alpha indirectly increase MAP kinase/AP-1 activation and MMP-1 production in UVA-irradiated dermal fibroblasts. *Chin Med J (Engl)*. 2006;119:827-831.
9. El-Domyati M, Attia S, Saleh F, et al. Intrinsic aging vs. photoaging: a comparative histopathological, immunohistochemical, and ultrastructural study of skin. *Exp Dermatol*. 2002;11:398-405.
10. Bhawan J. Assessment of the long-term safety of topical retinoids. *Cutis*. 2005;75(suppl 2):25-31.
11. Wulf HC, Sandby-Møller J, Kobayasi T, et al. Skin aging and natural photoprotection. *Micron*. 2004;35:185-191.
12. Campisi J. The role of cellular senescence in skin aging. *J Investig Dermatol Symp Proc*. 1998;3:1-5.
13. Al-Baker EA, Oshin M, Hutchison CJ, et al. Analysis of UV-induced damage and repair in young and senescent human dermal fibroblasts using the comet assay. *Mech Ageing Dev*. 2005;126:664-672.
14. Naru E, Suzuki T, Moriyama M, et al. Functional changes induced by chronic UVA irradiation to cultured human dermal fibroblasts. *Br J Dermatol*. 2005;153(suppl 2):6-12.

15. Ma W, Wlaschek M, Tantcheva-Poór I, et al. Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. *Clin Exp Dermatol*. 2001;26:592-599.
16. Glogau RG. Aesthetic and anatomic analysis of the aging skin. *Semin Cutan Med Surg*. 1996;15:134-138.
17. Calderone DC, Fenske NA. The clinical spectrum of actinic elastosis. *J Am Acad Dermatol*. 1995;32:1016-1024.
18. Gupta MA, Gilchrest BA. Psychosocial aspects of aging skin. *Dermatol Clin*. 2005;23:643-648.
19. Gendler EC. Topical treatment of the aging face. *Dermatol Clin*. 1997;15:561-567.
20. Stern RS. Clinical practice. treatment of photoaging. *N Engl J Med*. 2004;350:1526-1534.
21. Flynn TC, Coleman WP. Topical revitalization of body skin. *J Eur Acad Dermatol Venereol*. 2000;14:280-284.
22. Brieva A, Guerrero A, Pivel JP. Mecanismos bioquímicos y farmacológicos relacionados con la actividad de la secreción de *Cryptomphalus aspersa* (SCA) (Endocare®) en radiodermitis. *Dermatol Cosmet*. 2001;11:71-75.
23. Abad RF. Treatment of experimental radiodermatitis with a regenerative glucoproteic mucopolysaccharide complex. *Dermatol Cosmet*. 1999;9:53-57.
24. Brieva A, Philips N, Tejedor R, et al. Molecular basis for the regenerative properties of a secretion of the mollusk *Cryptomphalus aspersa*. *Skin Pharmacol Physiol*. 2008;21:15-22.
25. Glogau RG. Physiologic and structural changes associated with aging skin. *Dermatol Clin*. 1997;15:555-559.
26. Grove GL, Grove MJ, Leyden JJ. Optical profilometry: an objective method for quantification of facial wrinkles. *J Am Acad Dermatol*. 1989;21(3 pt 2):631-637.
27. Grove GL, Grove MJ, Leyden JJ, et al. Skin replica analysis of photodamaged skin after therapy with tretinoin emollient cream. *J Am Acad Dermatol*. 1991;25(2 pt 1):231-237.
28. Urbach F, Forbes PD, Davies RE, et al. Cutaneous photobiology: past, present and future. *J Invest Dermatol*. 1976;67:209-224.
29. Berardesca E, Cameli N, Primavera G, et al. Clinical and instrumental evaluation of skin improvement after treatment with a new 50% pyruvic acid peel. *Dermatol Surg*. 2006;32:526-531.
30. Shin JY, Song JY, Yun YS, et al. Immunostimulating effects of acidic polysaccharides extract of *Panax ginseng* on macrophage function. *Immunopharmacol Immunotoxicol*. 2002;24:469-482.
31. Meshel AS, Wei Q, Adelstein RS, et al. Basic mechanism of three-dimensional collagen fibre transport by fibroblasts. *Nat Cell Biol*. 2005;7:157-164.