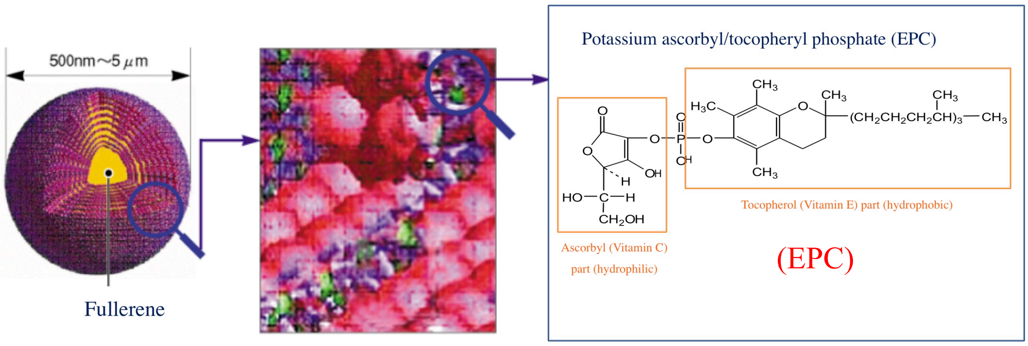
Nanosphia F (Amphiphilic Vitamin CE + Fullerene capsule)

Antioxidant and hair growth effects of fullerene Nano-capsule

What is "Nanosphere", anti-oxidative vitamin-C/E derivatives for nano-capsule materials?



(EPC) EPC is a Japanese quasi-drug raw material, it is effective in preventing dandruff and itch of scalp.

Ascorbyl (Vitamin C) part (hydrophilic)

- Amphiphilic character
- An inhibitory effect on the activity of skin-lipase relating to acne formation
- A suppressing effect on the growth of acne bacteria (P. acnes)
- Reactive-oxygen species scavenging effects on singlet oxygen, super-oxide anion radicals, and peroxide lipids
- A cell-turn-over improving ability
- A humectant ability

1. Introduction

Under normal conditions, L-Ascorbic acid (vitamin C) Ascorbic acid strong reducing effect and undergoes oxidization to become discolored when used with in drugs. Synthesis of ester derivatives with modification of carbon 2, the most reactive site of Ascorbic acid, has been performed in Japan since the 1960s, and many esters of Ascorbic acid are now used as food additives, stock feed additives, and cosmetic ingredients. Among the C2 ester derivatives of Ascorbic acid, the most commonly used worldwide is L-Ascorbic acid-2- phosphate ester (AP). AP were first developed in the 1970s as ingredients of medical cosmetics that prevent pigmentation, and their tyrosinase inhibitory effect prevents melanin production by the pigment cells of the skin. AP activates collagen production, and it has an effect to promote cell specialization. In addition, AP erases reactive oxygen species(ROS), it restrains lipids peroxidation. In this way AP protects a cell from ROS, and AP extends cell life time.

Industrial production of L- ascorbyl-2-phosphate-Tocophelol (EPC), an EPC with ester modification of Tocopherol of AP, started in Japan. AP are water soluble, but the lipid solubility of EPC was increased by modification of Tocopherol, a long chain of carbon, markedly improving uptake by skin tissue and cells. The structural formulae of Ascorbic acid, AP, and EPC are shown in

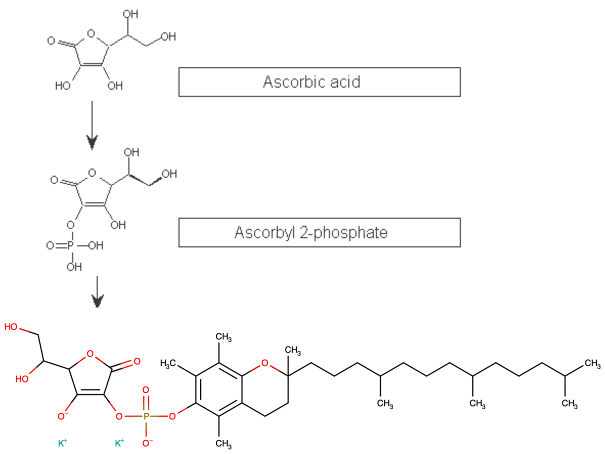


Fig.1.

Ascorbyl 2-Phosphate tocopherol, EPC is a novel vitamin C derivative, potacium salt of ascorbyl 2-phosphate tocopherol(EPC), newly designed Ascorbic acid amphiphilic derivative of ascorbyl 2-phosphate (AP), conjugated with a long carbon chain (Tocopherol) . AP has been proved very efficacious for skincare because of its capability of scavenging reactive oxygen species and promoting collagen synthesis. Thanks to its moderate hydro- phobicity, EPC penetrates effectively into dermis and is enzymatically converted to ascorbic acid quickly during permeation. EPC retain the stability of AP and in vivo Ascorbic acid activity, along with improved tissue permeability and intracellular delivery of

Ascorbic acid due to their reduced affinity for water. EPC have a strong surfactant effect because of being water soluble, a required property, as well as being lipophilic. EPC has an effect to restrain melanoma metastasis. Because EPC are strong antioxidants that are equivalent to Ascorbic acid and can be used Ascorbic acid film coating for microcapsules to allow the production of microcapsules with a free radical barrier, it has become increasing clear that their characteristics have various EPC phenomenon. In the present article, some of the interesting properties of EPC are discussed. The main effect of EPC was examined in detail. They are experimented on in order to evaluate EPC, and reported some effects of EPC.

2, Production of microcapsules using EPC

EPC are amphiphilic agents that form liquid crystals when combined with lipids as surfactants. Ascorbic acid result, lipids encapsulated by an EPC film can be produced. The liquid crystal structure of EPC is formed by multiple layers of water and lipid (i.e., water-lipid- water-lipid, etc.)(Fig.2.), so water-soluble materials can be mixed in the aqueous layers. Microcapsules made in this fashion become self-emulsifying (Picture) and addition of water leads to emulsification as dispersion (Picture). When the self-emulsifying process occurs, EPC in the form of microcapsules become dispersed in water Ascorbic anionic dispersion, so electrophoresis easily performed (Picture). Using this phenomenon, water-soluble materials with no electric charge, such as lipids, peptides, sugar chains, and antibodies, can be introduced as ions by iontophoresis.

We developed nano spheres for hair growth which subsumed fullerene. In the next chapter, we will explain the excellent hair growth effect of fullerene.

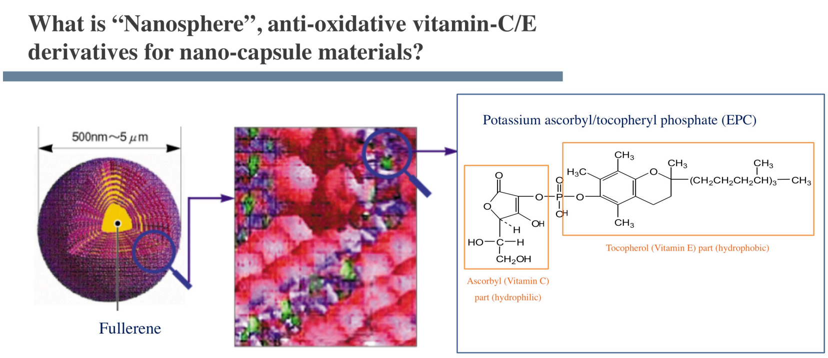


Fig.2. Multi-layer liquid crystal structure of self-emulsifying EPC + Fullerene nano/ microcapsules (Nanosphia) .

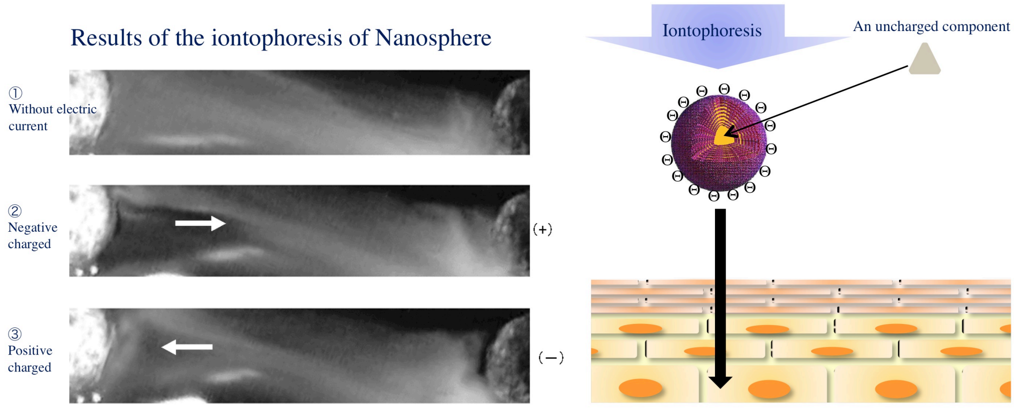
We have already succeeded in producing an EPC-based self-emulsifying encapsulated lipid preparation (Nansphia), in which CoQ10, astaxanthin, VCIP, and fullerene are encapsulated by EPC. With these EPC microcapsules, the stability of both the EPC themselves.

Below are three other interesting features of Nanosphia.

1) Ion introduction device can be used because liposoluble raw material can be ionically dispersed

2) Nanosphia permeates mainly from hair follicles into the skin, so that the components can be delivered efficiently to hair-related micro-organs
3) Nanosphia can deliver ingredients to skin tissue persistently over a long period of time compared with general skin penetration

Nanosphere can allow uncharged compounds to be iontophoresed.

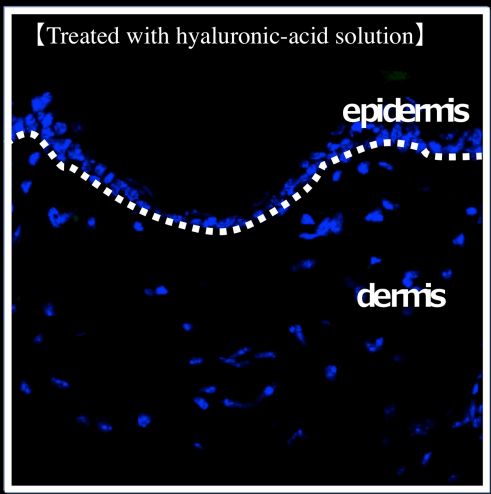


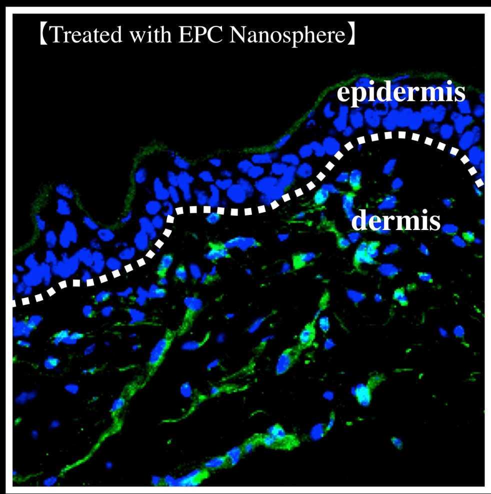
◆ Nanosphere, of which outer shell membrane is composed of vitamin-C derivative including APPS or EPC, is negatively charged.

◆ After being included in the core of Nanosphere, an uncharged component can be iontophoresed.

The observation of the distribution status of GFP with a confocal laser scanning microscope

In the photographs, the green and blue colors indicate GFP and cell nuclei, respectively.

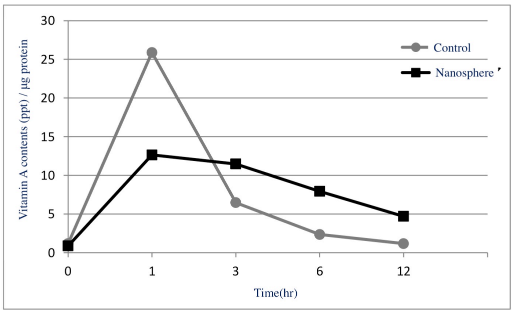




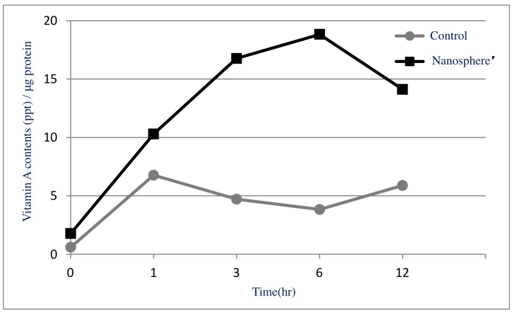
Nanosphere can allow high molecular-weight GFP to permeate into dermis.

Enhancement of the permeations of Nanosphere included components

EPIDERMIS



DERMIS



The permeation test of retinol derivative formula included in Nanosphere to three-dimensionally cultured cutaneous tissue model was performed.

As a result, retinol (RT) concentration in the tissue cultured with retinol derivative formula included in Nanosphere was found to be significantly lower than that of non-included sample at 1 h after removing formula($p < 0.01$).
In contrast, at 3~12 h, RT concentration in the cultured with retinol derivative formula included in Nanosphere was found to be significantly higher than that of non-included sample, and the longevity of RT in the tissue model was elongated further.

A sustained release function

Antioxidant and hair growth effects of fullerene

Fullerene is a molecule with a soccer ball-like structure consisting of 60 carbon atoms, and is said to be the third carbon allotrope (after graphite and diamond) (Fig 1). Owing to its unique structure, it is chemically characterised by strong antioxidation and is anticipated to have various applications.[1] It is already being sold as a cosmetic raw material because of its antioxidant properties. Several clinical studies have reported its efficacy in reducing spots, wrinkles, and acne, which are thought to be associated with oxidative stress.[2,3,4] Although it has been reported that oxidative stress is involved in alopecia areata,[5] the link between hair growth and oxidative stress has not been found; moreover the effects of substances with antioxidant properties, such as fullerene, are also unknown. Therefore, we conducted a single-blind clinical trial to determine how application of a tonic containing fullerene to human male scalps influences hair growth.

Fullerene as cosmetic raw material Discovery of fullerene

Fullerene was discovered during a study on interstellar molecules in 1985.[6] Discoverers won the Nobel Prize in Chemistry for his achievement in 1996. Fullerene is present not only in outer space but also on the Earth, and is found in Russian shungite and Chinese ink. Many researchers have turned their attention to fullerene because of its high electron-accepting ability and semiconducting properties, in addition to its unique soccer ball-like structure. They have examined a broader range of applications in life science (e.g., pharmaceutical products) and industrial materials (e.g., solar batteries) to achieve practical use. Since the 1990s, various studies have reported excellent antioxidant properties of fullerene. Researchers from DuPont presented a paper titled "C60 as a Radical Sponge" to describe how fullerene absorbed radicals like a sponge.[7] Fullerene as a raw material for antioxidant cosmetics was approved in 2005, and has been in use for 10 years or more.

Antioxidative effect of fullerene

Within the fullerene molecule, all carbon atoms are in their sp^2 hybridisation state and π electrons are delocalised around the frame of fullerene like a cloud. Two types of reactions are thought to be involved in its mechanism of antioxidation: an additive reaction by the addition of radicals to fullerene and a catalytic response in the exchange of electrons. Because fullerene shows no reduction effect with hydrogen atoms, unlike antioxidants such as vitamin C, antioxidative effect cannot be assessed via the DPPH assay, which is generally used for antioxidant evaluation; however, the antioxidative effect of fullerene can be confirmed through various other methods. In a study involving detection of hydroxyl radicals, generated by the Fenton reaction, using electron paramagnetic resonance (ESR) spectroscopy, with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin-trapping agent, it was found that signal strength was lowered by addition of polyvinylpyrrolidone (PVP)-wrapped fullerene and the hydroxyl radicals were removed by fullerene.[8]

In another study involving evaluation of the antioxidation of fullerene with beta-carotene bleaching, it was reported that fullerene exhibits antioxidative effects under various oxidation conditions, including ultraviolet rays, compared to a vitamin C derivative and vitamin E, which are commonly used as antioxidants in cosmetics.[9] Generally, natural antioxidants such as vitamins (e.g., vitamin C and E) and polyphenols are unstable due to exposure to temperature and light and are stabilised by derivatisation. On the contrary, the structure of fullerene is extremely stable and barely disintegrates, even under high temperature conditions (100°C or more). In addition, its stability under light and pH is exceptional. Based on these characteristics, fullerene may be considered an antioxidant substance with high stability.

Application of fullerene to cosmetics

Fullerene exhibits an excellent antioxidative effect. It takes the form of a black solid (powder) and is highly soluble in nonpolar solvents such as toluene; however, it is insoluble in polar solvents such as water or ethanol. Therefore, to achieve its practical use as a cosmetic raw material, it is necessary to facilitate its combination with cosmetics by solubilising or other procedures.

Currently, four types of fullerene are manufactured as cosmetic raw materials: water-dissolved PVP-wrapped fullerene clathrate with water soluble polymer polyvinylpyrrolidone (PVP) used as an additive in cosmetics and pharmaceutical products (brand name: Radical Sponge®); squalane-dissoあlved fullerene in which fullerene is dispersed into the hydrocarbon squalane (brand name: LipoFullerene®); liposome-precursor fullerene in which fullerene is dispersed into hydrogenated lecithin and soybean sterol (brand name Moist Fullerene®); silica-containing fullerene in which fullerene is dispersed into porous silica (brand name: Veil Fullerene®). All these products include C_{60} of 200 ppm or more and are contained in various kinds of cosmetics such as lotion, liquid cosmetics, and foundation.

Measures taken against reactive oxygen species to enhance beauty using fullerene

Ageing of the skin involves physiological ageing due to age and photoageing due to exposure to light. It is thought that light (ultraviolet rays) causes inflammation due to generation of reactive oxygen species, spots due to activation of melanocyte, and wrinkles due to destruction of collagen and disorders involving its repair (Fig 2).[10]

Fullerene removes reactive oxygen in the skin through its antioxidative effect and thus prevents skin problems. Once ultraviolet rays are irradiated to cultured cells and three-dimensional skin models, reactive oxygen species is generated, which induces the release of inflammatory cytokines.

It is known that these cytokines cause inflammation including erythema and stimulate melanocytes to promote melanin synthesis. In a study on anti-inflammatory effects of fullerene using a three-dimensional skin model, it was reported that the release of PGE2, a kind of inflammatory cytokine, increased by ultraviolet irradiation, was inhibited after fullerene addition.[11] In addition, various clinical trials have been performed, and reported on the above-mentioned whitening, anti-wrinkling, acne care, and normalising of the horny cell layer.[12]

Safety of fullerene

Because fullerene is a relatively new molecule, the findings of its safety were limited at the beginning. Therefore, in addition to safety testing of the study items, required for approval of quasi drug additives in Japan in 2004, we conducted a safety test on fullerene. Consequently, no test results indicated it to be hazardous.[13,14,15]

Although 11 years have passed since the launch of fullerene as a cosmetic raw material, there have been no reports of its harmful effects on the skin. Therefore, it may be said that fullerene is safe as a cosmetic raw material. Based on the knowledge accumulated for over 10 years, we believe that fullerene is a sufficiently safe cosmetic raw material.

Effect of fullerene on hair growth

There are fewer reports of the influence of reactive oxygen species on hair growth than on skin ageing due to oxidative stress. There are several reports of a link between oxidative stress and hair growth: sebum peroxide is associated with alopecia areata;[5] oxidation of sebum induces apoptosis of the hair papilla;[16] accumulation of hydrogen peroxide is linked to whitening of hair.[17]

According to these reports, it is surmised that antioxidant ingredients do have a certain effect.

However, there have been no studies on the effect of antioxidants such as fullerene on hair growth. We conducted a single-blind clinical trial to determine how application of a tonic containing fullerene to the human male scalp influences hair growth.

Method of experiment

A tonic containing fullerene combined with a base consisting of water and ethanol (2% Radical Sponge, 20% ethanol, and 5%-1,3-BG/purified water) was used as a test sample. A control tonic was prepared (20% ethanol, 5%-1,3-BG/purified water).

The study was conducted in accordance with the Helsinki Declaration. The subjects consisted of 16 adult men (in the age group of 30-50 years) who provided their consent to participate in this study. This study was performed with single blinding. Treatment for androgenetic alopecia (AGA) and the use of hair-growing agents, hair restorers, and hair growth stimulants were restricted among the subjects. The test areas were defined as the sites approximately 5 cm from the right and left ears.

The subjects were asked to apply a fullerene-containing tonic to the right or left specified test part and to apply a control tonic to the test part on the other side. Application was conducted two times (morning and evening) every day until the end of the study and after shampooing. The study period was set for 24 weeks. Assessment was performed before the study and at 12 and 24 weeks after the initiation of the study using a phototrichogram. In this study, we cut the hair and captured pictures of it at a certain area on the test portion (about 1 cm^2), and then took pictures of the same part after a few days.

The hair growth rate and anagen hair ratio for the duration of the study can be precisely measured by a comparison of photos before and after treatment. In this clinical trial, using a Folliscope (Lead M Co., Seoul, Korea), we took photos of the same portions just after haircut and at Day 3, and measured growth of each hair based on these photos to calculate hair growth per day (Fig 3). For hair density, a portion in which the number of hair strands can be precisely counted within the visual field was chosen.

We counted the number of hair strands at the designated portion and calculated the hair density of the portion per 1 cm^2 from the chosen area (Fig 4). In addition, diameter of 10 hair strands per test area was measured.

Results

Figure 5 shows the relative hair-growth speed of the part to which a fullerene tonic was applied in comparison with that of the part to which a control tonic was applied. There was no significant difference at 12 weeks after the initiation of the treatment; however, the hair-growth speed significantly increased by 16% at 24 weeks (paired t-test). As shown in Figure 6, no significant change by application of fullerene tonic was found in the hair density, the diameter of the hair, or the anagen hair ratio. No subject reported side effects throughout the study period; therefore, it is safe to use.

Discussion

It is known that oxidative stress on the tissue due to reactive oxygen species may be a major contributor to ageing. It has been reported that an accumulation of reactive oxygen species can be found in the hair follicle.[18] In addition, it has been suggested that ageing of the dermal papilla cell is induced by oxidative stress, which may lead to etiology of male pattern baldness.[19]

With the presence of oxidative stress in the hair loss portion of alopecia area, these findings may be considered evidence of oxidative stress being greatly associated with disturbance in hair growth and adjustment. In our study, fullerene as a scavenger of reactive oxygen species increased hair-growth speed. Considering that fullerene protects the cells from reactive oxygen species, our results in this study may be associated with fullerene-induced protection of the hair follicle cells.

Conclusion

Fullerene has been used in cosmetics to enhance the beauty of the skin of the face by primarily inducing whitening and reducing acne. However, based on our results, it has been found that fullerene can be expected to promote hair-growth by applying it on the scalp. Fullerene is expected to be used in hair tonics and liquid cosmetics for the scalp in the future.

References

1. Mashino T. Chemistry of fullerene as the third carbon allotrope [in Japanese], Gakkai Shuppan Center 1999; 224-230.
2. Ito M, Yamana S. Brightening effect caused by control of free radicals, *Personal Care* 2011; *12*(4), 44-47.
3. Kato S, Taira H, Aoshima H, Saitoh Y, Miwa N. Clinical evaluation of fullerene-C60 dissolved in squalane for anti-wrinkle cosmetics, *J Nanosci Nanotechnol* 2010; **10**: 6769–6774.
4. Inui S, Aoshima H, Nishiyama A, Itami S. Improvement of acne vulgaris by topical fullerene application: unique impact on skin care, *Nanomedicine* 2011; **7**(2), 238-241.
5. Arca E, Erbil H, Akay C, Sayal A, Gür AR. Antioxidant enzymes and lipid peroxidation in the scalp of patients with alopecia areata, *J. Dermatol. Sci.* 2002; **29**(2), 85-90.
6. Kroto HW, Heath JR, O'Brien SC, Curl RF, Smalley RE. C60: Buckminsterfullerene, *Nature* 1985; **318**, 162-163.
7. McEwen CN, McKay RG, Larsen BS. C60 as a radical sponge; *J. Am. Chem. Soc.* 1992; **114** (11): 4412-4414.
8. Xiao L, Takada H, Maeda K, Haramoto M, Miwa N. Antioxidant effects of water-soluble fullerene derivatives against ultraviolet ray or peroxylipid through their action of scavenging the reactive oxygen species in human skin keratinocytes. *Biomed Pharmacother.* 2005; **59**(7):351–358.
9. Takada H. Biosci. Biotechnol. *Biochem.* 2006; **70**(12), 3088-3039
10. Shimizu T. All about light dermatology [in Japanese], 34-36, Bunkodo, Tokyo, 2008.
11. Inui S, Mori A, Ito M, Hyodo S, Itami S. Reduction of conspicuous facial pores by topical fullerene: possible role in the suppression of PGE2 production in the skin, *J Nanobiotechnol.* 2014; **12**:6
12. Murakami M, Hyodo S, Fujikawa Y, Fujimoto T, Maeda K. Photoprotective effects of inclusion complexes of fullerenes with polyvinylpyrrolidone. *Photodermatol Photoimmunol Photomed.* 2013; **29**:196–203
13. Aoshima H, Saitoh Y, Ito S, Yamana S, Miwa N. Safety evaluation of highly purified fullernenes: based on screening of eye and skin damage. *J Toxicol Sci.* 2009; **34**:555–562.

14. Mori T, Takada H, Ito S, et al. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis, *Toxicology* 2006; **225**, 48-54.
15. Aoshima H, Yamana S, Nakamura S, Mashino T. Biological safety of water-soluble fullerenes evaluated using tests for genotoxicity, phototoxicity and pro-oxidant activity. *J Toxicol Sci 2010*; **35**: 401–409
16. Naito A, Midorikawa T, Yoshino T, Ohdera M. Lipid peroxides induce early onset of catagen phase in murine hair cycles. *Int J Mol Med.* 2008; **22**:725–729
17. Wood JM, Decker H, Hartmann H, et al. Senile hair graying: H2O2-mediated oxidative stress affects human hair color by blunting methionine sulfoxide repair, *FASEB J.* 2009; **23**(7), 2065-2075.
18. Sowden HM. Naseem KM, DJ Tobin. Differential expression of nitric oxide synthases in human scalp epidermal and hair follicle pigmentary units: implications for regulation of melanogenesis, *Br. J. Dermatol.* 2005; **153(**2), 301-309.
19. Bahta AW, Farjo N, Farjo B, Philpott MP. Premature senescence of balding dermal papilla cells in vitro is associated with pp16INK4a expression. *J Invest Dermatol* 2008; **128**: 1088–1094.