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CTAR®system, a hypoxia-induced and targeted anti-aging and repair system on human skin

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Abstract. – OBJECTIVE: The effective protection of skin cells and timely repair of damaged ones are of great significance for the maintenance of human skin's normal functions. Researches on skin cells mainly focus on the functions of repair factors. Relatively few studies have been made to clarify how skin cells sense the environment, absorb and transport nutrients, and achieve self-healing. CTAR®system, a new system, is proposed and demonstrated against human skin aging.

MATERIALS AND METHODS: This study is made based on the research outcomes of the 2013 and 2019 Nobel Prize in Physiology or Medicine: precise regulation of cellular vesicle transport networks, and cellular perception and responding mechanisms to oxygen. The technologies and methods derived from the award-winning theories were applied in the field of skin anti-aging.

RESULTS: It has shown that skin aging is a result of a series of injuries that have not been adequately repaired and then accumulate. The latest research on anti-aging of skin including channel identification, vesicle transport, induced activation, and damage repair has spawned CTAR®system, a hypoxia-induced and targeted anti-aging and repair system.

CONCLUSIONS: We determined that a considerable part of skin aging problems can be partially solved by taking full advantage of the vesicular trafficking system in skin and improving skin's metabolism levels and physiological functions under low oxygen levels CTAR®system.

Key Words:
CTAR®system, Vesicle transport, Hypoxia induction, Skin anti-aging.

Introduction

Skin aging, as the most sensible aging process of the human body, is a comprehensive result and external manifestation of overall aging of human functions[1]. Aging is an unavoidable natural law of human beings and starts at a very early stage, with individual divergences[2]. Traditionally, females aged 28 will see their peak physiological state in all aspects[3]; but the current medical theory claims that female skin aging begins at the age of 20 with processes such as glycosylation[4] and will gradually pass the deadline for physiological perfection. Then, aged skin will inevitably move towards rapid aging, and aging will further be accompanied by other hypofunctions in bones, teeth, hearing and so on[5]. Photoaging is considered an important factor in premature skin aging[6], which not only affects the normal function of skin and aesthetics, but also causes depression, inferiority and other psychological problems; all of those are even related to many serious diseases[7]. Therefore, it is of great importance to delay skin aging, especially in the early stage of skin aging.

Characteristics of skin Early Aging and its Molecular Mechanism

The initial aging of human skin is manifested in a variety of characteristics[8], and the macroscopic manifestations are that the skin begins to appear dry and rough, with reduced gloss and elasticity, increased fine lines, decreased internal water content and blood flow, reduced secretion of sweat and sebaceous glands, and increased skin allergy and irregular hyperpigmentation. There are three major in-depth changes for the above initial aging phenomenon. First, thinning of epidermis leads to skin sensitivity and pigmentation for that the viability of keratinocytes is declined[9], resulting in decreased repairing ability, after which the epidermis is damaged, and the barrier function of the skin gets weakened. Second, the increase of fine lines and dark lines and the decrease in skin elasticity occurs mainly due to the gradual decrease in the number of fibroblasts in the skin[10] and also the decline in the synthesis ability of collagen, elastin, and matrix.

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Third, the dehydration of cells leads to dry and rough skin given that skin's inherent moisture factors are gradually lost; and the external sebum secretion capacity is weakened, leading to a decrease in water retention capacity and a further decrease in water content[11]. How to effectively solve these problems is the key to the early anti-aging of skin.

Studies have shown that skin aging is a result of a series of injuries that have not been adequately repaired[12-14]. At present, people's understanding of skin aging has advanced to the level of cells and molecules. Insufficient supply of materials and energy to skin cells prevents various damages from being repaired in a timely manner, which is the main reason for the initial aging of skin. There are several vital biological processes involved in skin aging, such as dynamics and stability of DNA repair, mitochondrial function, cell cycle, apoptosis, extracellular matrix, lipid synthesis, and ubiquitin-induced proteolysis and cell metabolism[15]. When the supply of nutrients, growth factors and oxygen required for cell growth and repair is insufficient, the ability of human skin to repair and regenerate gradually weakens, and the skin loses vitality soon. How to realize the targeted distribution of substance and energy in these biological processes and realize the precise induction and activation of damaged and aging skin cells are crucial research topics for CTAR®system on skin anti-aging.

CTAR®system, a System for Precise Anti-aging

The CTAR®system (hereinafter referred to as CTAR®) covers the entire process of repair for skin cell damage (Figure 1). It integrates four phases including channel identification (Channel, C), vesicle transport (Transport, T), induced activation (Activation, A) and damage repair (Repair, R) to carry out the system and develops active formulations. Comprehensively investigating the structure and function of outer membrane of skin cells, the identification with opening of cell channels, and the transportation of the inner membrane like vesicles, CTAR® is used for the targeted transport of substances and energy that could penetrate into skin tissues, with the study of water channel assistance, nano-biotechnology and membrane fusion-repair technology. Comprehensively investigating the oxygen perception, hypoxia adaptation, oxidative stress, lipid peroxidation and mitochondrial damage of skin cells, combined with the methods of hypoxia induction, CTAR® is aimed to realize the precise induction and activation of damaged and aging skin cells. The development of its active formula is also based on this CTAR® hypoxia-induced and targeted anti-aging and repair system. Three research centers under The Japanese Academy of Dermatology (TJAD), each of which is led by a Nobel Prize Scientist, have suggested a combination of five major skin efficacy factors and aims to solve skin aging.

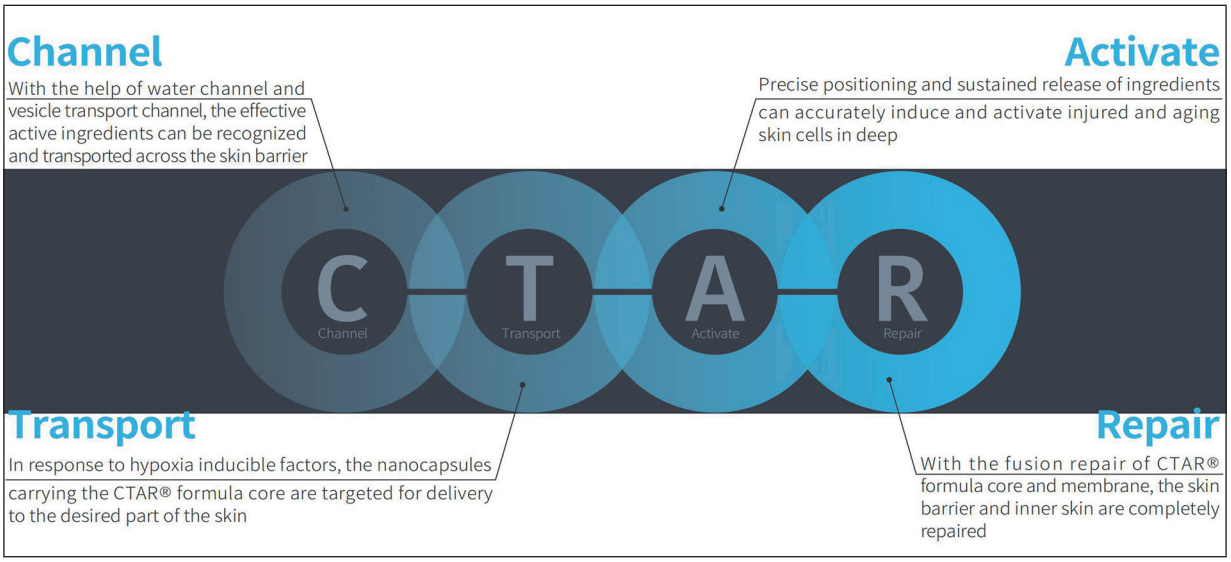

Figure 1. Schematic diagram of the principle of CTAR®system. The system consists of four parts including Channel, Activation, Transport and Repair. The detailed information of each part of the system is shown.

Mechanism of Targeted Induction and Activation

As an essential part of cell's inner membrane system, vesicular trafficking participates in the keeping of a steady state of endomembrane system and its composition[16]. Vesicle transport actively takes part in the fine regulation of skin aging and repairing process[17,18] – the metabolism, proliferation, and differentiation of skin cells, and the repair of wounds, all of which could not be separated from the inner membrane. The oxidative damage caused by poor cell respiration and increased oxidative stress on skin cells is a key factor for skin cell early aging[19]. It is also suggested that long-term hypoxia in skin cells will aggravate the generation of oxidative stress reactions and further cause an increase in active oxygen content[20], which in turn affects DNA structure, protease activity and collagen production, and accelerates the aging of the skin. Following these principles, researchers believe that a considerable part of skin aging problems can be solved to some degree by making use of the vesicular trafficking system in human skin and improving skin's metabolism levels and physiological functions under low oxygen.

Regardless of yeast or human, skin or nerve, the mechanism or principle of vesicle transport and membrane fusion is the same[21]. Proteins are first folded in the endoplasmic reticulum and then transported to the Golgi apparatus for modification and encapsulated by vesicles. Finally, the proteins are transported to designated positions to perform their physiological functions[3], which is an essential way of transport for a range of physiological activities, such as the transmission of neurotransmitters, the release of hormones, and the combination of proteases or cytokines with others[22]. Fries et al[23], Novick et al[24], and Südhof et al[25] took the lead in revealing the fine structure and regulatory mechanism of this intracellular transport system.

There are many theories about cell senescence, including the free radical hypothesis, mitochondrial hypothesis, and telomere theory, etc., all showing that aging is related to reactive oxygen species (ROS)[26]. Oxygen is required in intracellular redox reactions whilst too high or too low levels of oxygen both take a toll. As the skin micro-environment gets worse, cell respiration is blocked especially when cells stay in a hypoxic state for a long time. The content of ROS and free radicals will rise sharply, causing the cells to face peroxidative stress[19]. Semenza et al[27], Maxwell et al[28], and Ivan et al[29] were the first to reveal the molecular mechanism by which cells sense and adapt to oxygen levels and found that oxygen sensing systems exist in almost all tissues including skin, not just the kidney cells. Sensing of O_2 by keratinocytes in the epidermis was also found causing dynamics in cutaneous blood flow that affect the production of hormone erythropoietin, thereby modulating red blood cell production and the O_2-carrying capacity of blood[30]. These lay a solid foundation to understand the extent to which oxygen levels affect the skin's cellular metabolism and physiological functions.

Channel Identification and Transmembrane Transport

We know that cell membranes divide cells into individual spaces, serving as the basic structural and functional unit for vital activities[31]. The phospholipid bilayer acts as the basic skeleton of cell membrane and forms the physical barrier of cell with a small number of membrane proteins, sugars and glycolipids together[32]. Therefore, the entry of various nutrients and growth factors into human skin cells is often not free but needs to be achieved in many ways to cross the membrane. Transport of water channel, ion channel, exocytosis and endocytosis and vesicles are ways in which substances enter and exit the membrane[33]. The absorption of water and salt ions by skin cells needs to be achieved by carriers such as aquaporins and ion channel proteins[34]; and the absorption of some macromolecular substances, such as proteins, enzymes, ribose, and polysaccharides, needs to be completed through the cellular inner membrane system[35]. In addition, the excretion of metabolic waste, such as organelle debris and viral particles, also requires the membrane system to offer a channel[36].

Skin cells can absorb moisture from external environment directly[37]. Hyaluronic acid, as the main component of extracellular matrix, can maximize the openness of water channels, assisting to absorb water equivalent to 500-1000 times its own weight[38] for skin cells and promote skin metabolism, cell hydration, and elasticity maintenance, improving the vesicle transport efficiency of skin cells significantly. Low-molecular hyaluronic acid can promote the synthesis of collagen and ceramide and help the migration and proliferation of skin cells after being absorbed[39] while macromolecular hyaluronic acid has the ability to retain water, hydrate, and nourish the stratum

corneum on the skin surface. In addition, study has shown that hyaluronic acid can also regulate the activity of immune cells[40].

Additionally, the special properties of phospholipid bilayer of cell membrane make it easier for fat-soluble substances and uncharged small molecules such as microlipid vesicles and oxygen to recognize and penetrate into skin cells[32]. Cell membrane has a hydrophobic effect that high-fat-soluble substances diffuse easily while low- and non-fat-soluble substances are difficult to pass through. As a result, skin absorption of such substances gets worse. The process of microlipid capsule encapsulation can be used to divide phospholipids, aliphatic acid, etc. into nano-sized microlipid vesicles[41], which can effectively solve this problem. Liposomal microlipid capsules, as "*in vitro* vesicles", can encapsulate water-soluble and macromolecular substances in their aqueous cavities while lipid-soluble or facultative substances are combined in the lipid bilayer or its lipophilic group[42]. With the help of fusion of vesicles with cell membranes, various substances required for cell repair can be delivered into the skin[43].

Vesicle Transport and Membrane Fusion Repair

After nutrients and repair factors penetrate into skin cells, it actually arrives at the "main battlefield" of vesicular trafficking. Specifically, intracellular transport of vesicles includes donor generation (germination), transport, anchoring, and final fusion with the target membrane[16]. Their molecular regulatory mechanisms were already clarified. In vitro biochemical methods were used by Fries et al[23] to discover vesicle transport and protein interaction pathways between vesicles and target organelles with mammalian cells. Novick et al[24] used yeast as a model for the first time to find out a series of key genes necessary for intracellular vesicle transport and answered these questions at a genetic level.

When vesicle transport is disturbed, the internal environment of the skin will fall into a disordered state[44], which will develop a series of skin aging problems such as defects in the progress of skin cell development, cytochrome deposition and capillary dilation. James Rothman's group[45] is now dedicated to the research on biomedical fusion and ultra-high resolution Golgi dynamics; nanotechnology has also been applied to skin cells to improve the release rate of vesicle transport and the skin cell vitality. Randy Schekman's group[46] is trying to apply the theory of vesicular trafficking on stem cell research and biological artificial intelligence; the study on differentiation and transplantation of skin cells is promising for the solution of skin aging. Their work appears to merge in the study of large eddies.

Hypoxia Perception and Induced Activation

When nutrients are transported to specific targets of skin, how to accurately induce and activate damaged and aging skin cells is the next challenge. Cellular respiration of damaged or senescent skin cells ultimately slows down. This process is usually accompanied by a decrease in intracellular oxygen content. Wang et al[47] found that genes regulated by hypoxia-inducible factor 1 (HIF-1) can affect mitochondrial respiration and regulate the cellular adaptive response to hypoxia. This cellular hypoxic adaptive response makes it possible for skin cells to maintain their metabolism in a hypoxic environment. Therefore, the application of hypoxia-inducible methods which can capture hypoxia signals allows various nutrients and growth factors upstream to be precisely delivered into such skin cells and act on for induction and activation, so that the substances and energy used for cell repair can be efficiently used.

Long-term hypoxia may cause skin oxidative stress, mitochondrial dysfunction, mitochondrial autophagy, microglial activation and other physiological and pathological processes[48]. HIF-1 can regulate vascular endothelial growth factor and promote the generation of new blood vessels around skin tissues, thus achieving the restoration of skin blood vessels and bringing sufficient nutrients and gas exchange for impaired and senescent cells. At present, Semenza's group is studying the molecular mechanism of oxygen homeostasis and the molecular mechanism of angiogenesis and vascular remodeling[49]. With an application of gene and stem cell therapy of severe limb ischemia and skin burns based on a mouse model, they aim to find more applications in the case of human skin cell activation. The precise induction and activation of injured and aging skin cells also form an important part of CTAR®.

Growth Factors and Skin Damage Repair

After substances being transported by vesicles and after the process being induced by hypoxia, skin cells start to utilize upstream nutrients and growth factors for damage repair. Nutrients and

factors often include amino acids[50], vitamins[51], ceramides[52], coenzyme Q[53], plant extracts[54,55] and so on, among which vitamins play an important role. Selenoprotein is a component of most antioxidant enzymes while vitamin A can promote the expression of selenoprotein coding gene; vitamin A can also combine with organic peroxyl radicals, blocking oxidation reaction and delaying aging of skin cells[56]. Telomere is synthesized by telomerase and its shortening is a sign of skin aging. Nomura et al[57] have shown that tocopherols, vitamin E compounds, can enhance telomerase activity, thereby extending the length of telomeres and delaying cell aging.

In addition to vitamins, ceramide and coenzyme Q also play important roles. Ceramide is the central link of sphingomyelin metabolism. After *de novo* synthesis and sphingomyelin circulation, it can be secreted from the epidermal layer through lamellar bodies to the stratum corneum and form intercellular components[58]. Ceramide, as the second messenger of lipid, is a key signaling molecule in the intracellular oxidative stress pathway, which regulates cell differentiation and induces apoptosis. Coenzyme Q10 is the only coenzyme Q that exists in human body. It acts as an important hydrogen transporter in the respiratory chain of skin cells, participating in the process of oxidative phosphorylation and ATP production, regulating the cellular redox environment[59]. The content of coenzyme Q10 declines with age and needs to be supplemented for adults.

Targeted Regulation Based on Encapsulation

Vesicles are important for intracellular and intercellular substance exchange and signal transmission; they are also considered to be good natural wrapping and transporting materials for transporting active ingredients across skin barrier and cytomembrane[60]. Microencapsulated liposomes with nano-sized diameter and vesicle grade have been very similar to vesicles and play a role. Cells in both surface and deep layers of the skin can sense oxygen, and the keratinocytes on the surface can respond to the external environment under low oxygen conditions[30]. Therefore, the hypoxia-induced regulation technology based on encapsulation could be used to achieve precise induced activation and targeted repair of injured and aging skin cells.

The research centers, led by Rothman, Schekman and Semenza, suggested CTAR®, containing five major efficacy factors that are capable of improving the condition of human skin through vesicle transport and hypoxia induction system. CTAR® takes use of natural moisturizing factors to promote water channels and regulate skin's basic metabolism and then transfers nano-microlipid capsules that carry four anti-aging factors (tissue repair factor, antioxidant factor, cell-activity promoting factor, and hypoxia-inducible regulator) into skin. These factors further activate and repair injured or aging skin cells through the HIF-1 regulated pathway. This further strengthens the communication ability of skin cells, protecting them from free radical damage and promoting collagen production, aiming to solve the skin oxidative stress and to fight against skin aging.

Conclusions

In summary, the latest research on skin cell activation and anti-aging based on vesicle transport and hypoxia induction theory shows that Rothman, Schekman, Semenza and TJAD proposed the CTAR®system, a hypoxia-induced and targeted anti-aging and repair system, which felicitously divided skin anti-aging and repair process into four stages. The CTAR®system takes advantages of scientific formulas, ratios and vesicle grade nano-encapsulation technique for which the nutrients and growth factors required for skin repair are transported to the deep of skin, achieving precise activation and repair for injured and senescent skin cells through precise regulation of vesicle transport and hypoxia induction; meanwhile, the absorption and utilization efficiency of nutrients by skin is significantly improved, resulting in excellent effectiveness of which traditional repair methods cannot compare with. The proposal of this theory has opened up a brand-new vision to solve the problem of human skin aging.

Conflict of Interest

Shinichiro Akiyama is a scientific director under The Japanese Academy of Dermatology (TJAD), the association that has developed the CTAR®system.

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References

1) Proksch E, Brandner JM, Jensen JM. The skin: an indispensable barrier. Exp Dermatol 2008; 17: 1063-1072.
2) Montagna W, Carlisle K. Structural changes in aging human skin. J Invest Dermatol 1979; 73: 47-53.
3) Harman D. Free radical theory of aging. Mutat Res 1992; 275: 257-266.
4) Danby FW. Nutrition and aging skin: sugar and glycation. Clin Dermatol 2010; 28: 409-411.
5) Madison KC. Barrier function of the skin: "la raison d'être" of the epidermis. J Invest Dermatol 2003; 121: 231-241.
6) Skotarczak K, Osmola-Mańkowska A, Lodyga M, Polańska A, Mazur M, Adamski Z. Photoprotection: facts and controversies. Eur Rev Med Pharmacol Sci 2015; 19: 98-112.
7) Uitto J. Understanding premature skin aging. N Engl J Med 1997; 337: 1463-1465.
8) Yaar M. Skin aging. Springer, 2006.
9) Wulf HC, Sandby-Møller J, Kobayasi T, Gniadecki R. Skin aging and natural photoprotection. Micron 2004; 35: 185-191.
10) Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 2006; 168: 1861-1868.
11) Verdier-Sévrain S, Bonté F. Skin hydration: a review on its molecular mechanisms. J Cosmet Dermatol 2007; 6: 75-82.
12) Yin D, Chen K. The essential mechanisms of aging: Irreparable damage accumulation of biochemical side-reactions. Exp Gerontol 2005; 40: 455-465.
13) Terman A, Brunk UT. Oxidative stress, accumulation of biological'garbage', and aging. Antioxid Redox Signal 2006; 8: 197-204.
14) Wang-Michelitsch J, Michelitsch T. Aging as a process of accumulation of Misrepairs. arXiv preprint arXiv:1503.07163, 2015.
15) Zouboulis CC, Makrantonaki E. Clinical aspects and molecular diagnostics of skin aging. Clin Dermatol 2011; 29: 3-14.
16) Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, Rammner B, Gräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R. Molecular anatomy of a trafficking organelle. Cell 2006; 127: 831-846.
17) Otlatıcı G, Yeğen G, Güngör S, Aksu B.Overview on nanotechnology based cosmeceuticals to prevent skin aging. Journal of the Faculty of Pharmacy of Istanbul University 2018; 48: 55-63.
18) Ferreira AF, Gomes DA. Stem cell extracellular vesicles in skin repair. Bioengineering (Basel) 2019; 6: 4.
19) Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. Oxidative stress in aging human skin. Biomolecules 2015; 5: 545-589.
20) Yang C, Ling H, Zhang M, Yang Z, Wang X, Zeng F, Wang C, Feng J. Oxidative stress mediates chemical hypoxia-induced injury and inflammation by activating NF-κb-COX-2 pathway in HaCaT cells. Mol Cells 2011; 31: 531-538.
21) Rothman JE. Lasker Basic Medical Research Award. The machinery and principles of vesicle transport in the cell. Nat Med 2002; 8: 1059-1062.
22) Chang CW, Chiang CW, Jackson MB. Fusion pores and their control of neurotransmitter and hormone release. J Gen Physiol 2017; 149: 301-322.
23) Fries E, Rothman JE. Transport of vesicular stomatitis virus glycoprotein in a cell-free extract. Proc Natl Acad Sci U S A 1980; 77: 3870-3874.
24) Novick P, Schekman R. Secretion and cell-surface growth are blocked in a temperature-sensitive mutant of Saccharomyces cerevisiae. Proc Natl Acad Sci U S A 1979; 76: 1858-1862.
25) Südhof TC. The synaptic vesicle cycle: a cascade of protein-protein interactions. Nature 1995; 375: 645-653.
26) Yaar M, Gilchrest BA. Cellular and molecular mechanisms of cutaneous aging. J Dermatol Surg Oncol 1990; 16: 915-922.
27) Semenza GL, Nejfelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. Proc Natl Acad Sci U S A 1991; 88: 5680-5684.
28) Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC, Pugh CW, Maher ER, Ratcliffe PJ. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999; 399: 271-275.
29) Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, Salic A, Asara JM, Lane WS, Kaelin WG Jr. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 2001; 292: 464-488.
30) Semenza GL. O2 sensing: only skin deep? Cell 2008; 133: 206-208.
31) Bretscher MS. The molecules of the cell membrane. Sci Am 1985; 253: 100-109.
32) Divecha N, Irvine RF. Phospholipid signaling. Cell 1995; 80: 269-278.
33) Saier MH Jr, Reddy VS, Tamang DG, Västermark A. The transporter classification database. Nucleic Acids Res 2014; 42: D251-D258.
34) Yool AJ, Campbell EM. Structure, function and translational relevance of aquaporin dual water and ion channels. Mol Aspects Med 2012; 33: 553-561.
35) Gropper SS, Smith JL. Advanced nutrition and human metabolism. Cengage Learning, 2012.
36) Krause WJ. Krause's essential human histology for medical students. Universal-Publishers, 2005.

37) Borgnia M, Nielsen S, Engel A, Agre P. Cellular and molecular biology of the aquaporin water channels. Ann Rev Biochem 1999; 68: 425-458.

38) Baumann L. Skin ageing and its treatment. J Pathol 2007; 211: 241-251.

39) Katsnelson A. Cosmetics: molecular beauty. Nature 2015; 526: S4-S5.

40) Litwiniuk M, Krejner A, Speyrer MS, Gauto AR, Grzela T. Hyaluronic acid in inflammation and tissue regeneration. Wounds 2016; 28: 78-88.

41) Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci 2015; 10: 81-98.

42) Bergers JJ, ten Hagen TL, van Etten EW, Bakker-Woudenberg IA. Liposomes as delivery systems in the prevention and treatment of infectious diseases. Pharm World Sci 1995; 17: 1-11.

43) El Maghraby GM, Barry BW, Williams AC. Liposomes and skin: from drug delivery to model membranes. Eur J Pharm Sci 2008; 34: 203-222.

44) Rinnerthaler M, Streubel M K, Richter K. Molecular Mechanisms of Skin Aging and Age-Related Diseases. CRC Press, 2016.

45) Huang F, Sirinakis G, Allgeyer ES, Schroeder LK, Duim WC, Kromann EB, Phan T, Rivera-Molina FE, Myers JR, Irnov I, Lessard M, Zhang Y, Handel MA, Jacobs-Wagner C, Lusk CP, Rothman JE, Toomre D, Booth MJ, Bewersdorf J. Ultra-high resolution 3D imaging of whole cells. Cell 2016; 166: 1028-1040.

46) Schekman R. Stem Cell Research: Opportunities and Challenges. Bulletin of the American Academy Spring, 2006: 17.

47) Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. Proc Natl Acad Sci U S A 1995; 92: 5510-5514.

48) Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, Gonzalez FJ, Semenza GL. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem 2008; 283: 10892-10903.

49) Samanta D, Prabhakar NR, Semenza GL. Systems biology of oxygen homeostasis. Wiley Interdiscip Rev Syst Biol Med 2017; 9: e1382.

50) Lintner K. Peptides, amino adds and proteins in skin care. Cosmet Toiletries 2007; 122: 26-34.

51) Lupo MP. Antioxidants and vitamins in cosmetics. Clin Dermatol 2001; 19: 467-473.

52) Coderch L, López O, de la Maza A, Parra JL. Ceramides and skin function. Am J Clin Dermatol 2003; 4: 107-129.

53) Prahl S, Kueper T, Biernoth T, Wöhrmann Y, Münster A, Fürstenau M, Schmidt M, Schulze C, Wittern KP, Wenck H, Muhr GM, Blatt T. Aging skin is functionally anaerobic: importance of coenzyme Q10 for anti aging skin care. Biofactors 2008; 32: 245-255.

54) Chen YH, Huang L, Wen ZH, Zhang C, Liang CH, Lai ST, Luo LZ, Wang YY, Wang GH. Skin whitening capability of shikimic acid pathway compound. Eur Rev Med Pharmacol Sci 2016; 20: 1214-1220.

55) Tsuchiya T, Fukui Y, Izumi R, Numano K, Zeida M. Effects of oligomeric proanthocyanidins (OPCs) of red wine to improve skin whitening and moisturizing in healthy women–a placebo-controlled randomized double-blind parallel group comparative study. Eur Rev Med Pharmacol Sci 2020; 24: 1571-1584.

56) Kartha VN, Krishnamurthy S. Antioxidant function of vitamin A. Int J Vitam Nutr Res 1977; 47: 394-401.

57) Nomura SJ, Robien K, Zota AR. Serum Folate, Vitamin B-12, Vitamin A, γ-Tocopherol, α-Tocopherol, and Carotenoids Do Not Modify Associations between Cadmium Exposure and Leukocyte Telomere Length in the General US Adult Population. J Nutr 2017; 147: 538-548.

58) Masukawa Y, Narita H, Shimizu E, Kondo N, Sugai Y, Oba T, Homma R, Ishikawa J, Takagi Y, Kitahara T, Takema Y, Kita K. Characterization of overall ceramide species in human stratum corneum. J Lipid Res 2008; 49: 1466-1476.

59) Knott A, Achterberg V, Smuda C, Mielke H, Sperling G, Dunckelmann K, Vogelsang A, Krüger A, Schwengler H, Behtash M, Kristof S, Diekmann H, Eisenberg T, Berroth A, Hildebrand J, Siegner R, Winnefeld M, Teuber F, Fey S, Möbius J, Retzer D, Burkhardt T, Lüttke J, Blatt T. Topical treatment with coenzyme Q10-containing formulas improves skin's Q10 level and provides antioxidative effects. Biofactors 2015; 41: 383-390.

60) Lohani A, Verma A. Vesicles: Potential nano carriers for the delivery of skin cosmetics. J Cosmet Laser Ther 2017; 19: 485-493.