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EXPERT OPINION

Polycomponent mesotherapy formulations for the treatment of skin aging and improvement of skin quality

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Abstract: Skin aging can largely be attributed to dermal fibroblast dysfunction and a decrease in their biosynthetic activity. Regardless of the underlying causes, aging fibroblasts begin to produce elements of the extracellular matrix in amounts that are insufficient to maintain the youthful appearance of skin. The goal of mesopreparations is primarily to slow down and correct changes in skin due to aging. The rationale for developing complex polycomponent mesopreparations is based on the principle that aging skin needs to be supplied with the various substrates that are key to the adequate functioning of the fibroblast. The quintessential example of a polycomponent formulation – NCTF® (New Cellular Treatment Factor) – includes vitamins, minerals, amino acids, nucleotides, coenzymes and antioxidants, as well as hyaluronic acid, designed to help fibroblasts function more efficiently by providing a more optimal environment for biochemical processes and energy generation, as well as resisting the effects of oxidative stress. In vitro experiments suggest that there is a significant increase in the synthetic and prophylactic activity of fibroblasts with treated NCTF, and a significant increase in the ability of cells to resist oxidative stress. The current article looks at the rationale behind the development of polycomponent mesopreparations, using NCTF as an example.

Keywords: mesotherapy, skin aging, skin quality

Introduction

Our knowledge of skin as a complex, immune, multifunctional organ is constantly evolving, including our insights into the skin aging process.[1–3] Recent histological, biochemical and biomolecular evidence has broadened our understanding of skin cell function and aging and provided new information on cell-to-cell interactions and particular features of intermolecular transport and communication.[4–7] This has provided an important stimulus to the development of new mesotherapy solutions as anti-aging treatments. Mesotherapy is a technique that involves micro-injections of therapeutic substances, such as hyaluronic acid, vitamins, minerals, and amino acids into the superficial papillary dermis of the skin.[8] This allows active and essential ingredients to come directly into contact with the dermal fibroblast cells that are key to the more favorable appearance of younger skin, and (in theory) have a beneficial effect on metabolic processes.

The range of available mesotherapy solutions widens choice for the practitioner, but also presents them with a challenge. In addition to having a good fundamental knowledge of dermatology and cosmetology, the practitioner may also benefit from an understanding of the physiological effects of the individual components of a particular formulation. Such knowledge should help to demystify the rationale behind the

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complex composition of polycomponent mesotherapies and aid judicious therapeutic choices.

Mechanisms of skin aging – the key role of fibroblasts

Skin aging can be due to intrinsic natural genetically determined factors, as well as extrinsic lifestyle-driven and environmental factors.[9,10] It is characterized by a number of objective physical indicators, including skin dryness, laxity and poor elasticity, color and surface irregularity, formation of pronounced skin markings, and wrinkles of different intensity. The processes underlying these features include a whole range of physiological changes, including both structural and biochemical alterations, as well as changes in neurosensory perception, permeability, response to injury, and repair capacity.[3]

Fibroblasts represent the major skin type in the dermis, where they have a key role in producing and maintaining the extracellular connective tissue that is crucial to maintaining the youthful appearance of skin.[10] Fibroblast dysfunction and a reduction in fibroblast biosynthetic activity are major factors involved in the skin aging processes. With aging, there is a decrease in the number of fibroblasts and a decrease in fibroblast production of hyaluronic acid, collagen, and other components of the extracellular matrix, as well as an increase in production of enzymes responsible for collagen fragmentation.[3,11–14] Fibroblast "collapse" also occurs due to loss of mechanical interactions with the surrounding extracellular matrix.[11,15,16] The exact mechanisms underlying the development of dysfunction in aging fibroblasts remain unclear, but age-related increases in oxidative stress, due to alterations in the balance between production and elimination of reactive oxygen species, may be one important contributor.[17,18]

Disrupted paracrine interactions between fibroblasts in the dermis and other cell types in the skin (especially keratinocytes located more superficially in the epidermis) may also play a key role in the skin aging process.[6,19–21] Paracrine cross-talk between keratinocytes and dermal fibroblasts appears to be involved in the modulation of collagen production and degradation via effects on MMP enzymes.[6] Such interactions may be particularly important in ultraviolet B (UVB)-induced skin aging, as UVB has only limited penetration into the dermis.[19] Exposure to UVB stimulates epidermal keratinocytes to secrete a whole range of pro-inflammatory cytokines that can interact with dermal fibroblasts.[21] For instance, UVB-induced secretion of IL-1α and GM-CSF has been shown to stimulate dermal fibroblasts to express elastase, an enzyme involved in extracellular matrix degradation that has been linked to loss of skin elasticity and wrinkle formation.[19–21]

Targeting the fibroblast using mesotherapy

Importantly, recent evidence suggests that dysfunctional fibroblasts in aged human skin retain capacity for functional activation, thus showing that fibroblasts are a viable target for anti-aging treatments.[16] Based on the current understanding of aging mechanisms in the skin, the development of mesotherapy solutions has focused on the identification of biomolecules that can improve (or help to maintain) fibroblast function and improve extracellular matrix component biosynthesis.[22]

Several basic principles have been key motivating factors behind the choice of components in mesotherapy formulations and these remain unchanged despite all the recent findings on fibroblast metabolism. Firstly, it is proposed that fibroblasts must have a sufficient supply of substrates in order to perform their biosynthetic activity. Accordingly, some mesotherapy solutions contain "construction materials", such as amino acids and nucleosides. An abundant supply of these materials may be important in aged skin, as substrate transport appears to be impaired in aging cells.[23] Secondly, most synthesis reactions are energy-consuming, so it is proposed that fibroblasts must have sufficient energy generation potential for synthetic activity. As put forward in the "defective powerhouse model" of skin aging, fibroblasts appear to have reduced energy generation as they age, so it may be important to maximize generation potential by supplying sufficient energy substrates.[24] However, it should be acknowledged that both these proposals have not been thoroughly investigated.

In addition to these two principles, it is becoming increasingly evident that the optimal function of fibroblasts is critically dependent on their reciprocal mechanical interactions with their surrounding microenvironment (in addition to biochemical signals).[11,15] Mechanical tension between fibroblasts and the surrounding extracellular matrix appears to be critical for fibroblast function and normal balanced production of collagen and collagen-degrading enzymes.[11,15] In aged skin, the use of dermal fillers appears to have a volume effect that induces mechanical tension in the dermal extracellular matrix and stimulates fibroblasts to produce collagen.[11]

The need for polycomponent mesotherapy

Cognizant of the rationale for targeting dysfunctional fibroblasts, a skin anti-aging mesotherapy solution should contain substances that create a favorable environment for optimal dermal fibroblast cell function. Based on this approach, a whole range of micronutrients and biomolecule substances have been considered for inclusion in various formulations and, at first glance, some may appear to be unduly complex.[8,25] For instance, NCTF135HA developed by Filorga Laboratories contains a total of 53 components, including vitamins, amino acids, minerals, coenzymes, and nucleotides, plus high-molecular weight hyaluronic acid (Table 1).[25]

It should also be noted that putting these multiple components together is not enough to guarantee their actual efficiency, as they need to maintain their stability and integrity in the formulation. For instance, the NCTF135 HA solution is sterilized by double filtration, and not by autoclaving, in order to preserve the vitamins and hyaluronic acid, which are particularly fragile and sensitive to heat.[25] In autoclaving, saturated steam under pressure at a temperature of 120°C causes protein denaturation by partial hydrolysis of the peptide chains and destruction of most vitamins.

The rationale behind these complex formulations lies in a thorough understanding of the functions that each ingredient has in skin-related biochemical processes, as outlined in the following sections.

Table 1 Constituents of the polycomponent mesotherapy NCTF®

Class	Component
Vitamins and vitamin-like substances	Vitamin A (retinol), Vitamin C (ascorbic acid), Vitamin E (tocopherol), Inositol B vitamins: Vitamin B1 (thiamine), B2 (riboflavin), B3 (nicotinamide), B5 (pantothenic acid), B6 (pyridoxine), B8 (biotin), B9 (folic acid), B10 (P-aminobenzoic acid), B12 (cyanocobalamin)
Minerals	Calcium chloride, Potassium chloride, Magnesium sulfate, Sodium acetate, Sodium chloride, Sodium dihydrogen phosphate
Amino acids	Alanine, Arginine, Asparagine, Aspartic Acid, Cystine, Glutamine, Glutamic Acid, Glycin, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Ornithine, Phenylalanine, Proline, Serine, Taurine, Threonine, Tryptophan, Tyrosine, Valine
Nucleosides	Deoxyadenosine, Deoxycytidine, Deoxyguanosine, Deoxythymidine, Methylcytosine
Co-enzymes	TPP (co-carboxylase), CoA, FAD, NAD, NADP, UTP
Other antioxidants	Glutathione
Hyaluronic acid	Non-reticulated sodium hyaluronate

Note: Data from NCTF135,135HA product information.[25]
Abbreviation: NCTF, New Cellular Treatment Factor.

Vitamins and vitamin-like substances

A number of vitamins have important functions that are relevant to the skin aging process.[26,27] Vitamin C (ascorbic acid) is an important antioxidant that speeds up DNA synthesis and is essential for collagen synthesis. Vitamin A (retinol) is another important vitamin with antioxidant effects that regulates epidermis regeneration and melanocyte activity, and also controls oil gland activity. Along with vitamin C, it assists in the synthesis of collagen and other intercellular matrix components. Vitamin E (tocopherol) is another important component with a high level of antioxidant activity. It controls skin physiological regeneration and starts repair processes in the case of skin damage. Inositol is a vitamin-like substance used by cells as a signaling molecule and contributes to the regulation of some important indicators of cellular homeostasis, such as the concentration of intracellular Ca^{2+}, and maintaining the capacity of the cell membrane.

The B vitamins are involved in the control of many cell functions, basically acting as coenzymes.[26,27] Vitamin B1 (thiamine) plays a key role in producing energy from carbohydrates and in obtaining ribose and deoxyribose from glucose, which are used for DNA and ribonucleic acid (RNA) synthesis. This vitamin also catalyzes the decarboxylation of alpha-keto acids (lactic and pyruvic), easing the cells' fight with metabolic acidosis. Vitamin B2 (riboflavin) and its derivatives (flavin adenine dinucleotide and flavin mononucleotide) are involved in the delivery of energy from carbohydrates and fat, as well as supporting redox cell metabolism and the activation of vitamins B6 and B9. Vitamin B3 (nicotinamide) is incorporated into two coenzymes (NAD and NADP), that play a crucial role in many reactions involving energy production from carbohydrates, fats and proteins, and in the biosynthesis of various molecules, such as fatty acids. Vitamin B5 (pantothenic acid) is a key part of the CoA molecule and is also essential in the generation of energy from carbohydrates, fats and proteins, and the synthesis of various biomolecules. Vitamin B6 (pyridoxine) is converted to a coenzyme (PLP) that is key in the cellular metabolism of amino acids, including their transfer through the cell membrane and intracellular transformation. Vitamin B7 (biotin; also known as B8) is used in four carboxylase enzymes that take part in regulating the metabolism of protein, fat and carbohydrates, and also has high anti-seborrheic activity. Vitamin B9 (folic acid) is necessary for cell division, due to its ability to transfer one-carbon fragments involved in the synthesis of DNA and RNA, and also contributes to the mutual transformation of amino acids. Vitamin B12 (cyanocobalamin) contributes to the metabolism of carbohydrates, proteins and fats, and participates in the formation of coenzyme forms of folic acid (ie, activation of vitamin B9).

Amino acids and related compounds

These represent the relevant substrates required to build dermal extracellular matrix proteins, mainly collagens.[26]

Minerals

Calcium is the main ion used to regulate cell homeostasis. Phosphorus is essential for cell wall generation and other biological membranes. Magnesium is required to maintain numerous normal enzymatic reactions (more than 180).[26]

Nucleosides

Five nucleosides are necessary to replicate DNA for fibroblast fission and RNA generation in the process of protein synthesis.

Coenzymes

Coenzymes are biochemical reaction catalyzers (see previous section on vitamins).[26,27] A cell is able to synthesize the majority of these coenzymes independently using vitamins. However, since a cell will have to spend a considerable amount of its own substrates and energy at the initial stages of synthesis, it is useful to include ready-made primary coenzymes in a formula to make fibroblast metabolism easier. Another important coenzyme is UTP, which assists in RNA generation.

Other antioxidants

In addition to the vitamin antioxidants (see earlier section on vitamins), tripeptide glutathione ranks among the most efficient endogenous antioxidants. Antioxidative properties are incidental both to the reduced form of glutathione and to the three enzymes that contain glutathione as cofactors: GPX, GR, and GST. Glutathione and glutathione-containing enzymes neutralize free radicals and peroxide compounds formed as a result of an uncontrolled oxidation process. There is an opinion that premature cell aging and apoptosis are very closely related with a reduction in glutathione intracellular concentration.[28–31]

Hyaluronic acid

Hyaluronic acid can accumulate and retain 1,000 times its weight in water, which may help the skin remain hydrated.[32] It also has anti-inflammatory, antibacterial, antifungal, and antioxidant properties.[33,34] In aged skin, hyaluronic acid production by fibroblasts is attenuated. Hyaluronic acid alone, or combined with vitamin cocktails (eg, NCTF-135HA), has been shown to maintain human skin fibroblast cell proliferation.[35] Furthermore, intradermal injection of hyaluronic acid has been shown to stimulate production of new undamaged collagen.[11,16] In part, this may relate to a mechanical effect on the fibroblast.[11,16] Data based on skin-targeted ultrasound also suggests that multiple hyaluronic acid micro-injections can be effective in women with signs of moderate photo-aging in the hands.[36,37]

Safety of mesotherapy

Cosmetic facial soft-tissue injection procedures, in general, have a very good safety profile.[38–41] Short-term effects, such as mild pain, redness, swelling, and bruising are relatively common and an expected consequence of the injections themselves, but true complications are rare, especially with formulations based on "non-permanent" filler materials, such as hyaluronic acid.[38–41] In a recent 5-year retrospective review of 2,089 injectable soft-tissue facial filler treatments performed at a single center in the United States (including 1,047 with hyaluronic acid-based formulations), true complications were reported in less than 1% of procedures (four cases of cellulitis, seven cases of nodule or granuloma formation, one case of skin necrosis, and two events defined as "other").[40] Complications were particularly uncommon among the patients receiving hyaluronic acid-based treatment, with only two events (0.2% of procedures) reported (one nodule/granuloma formation, one "other").[40] Complications were more frequent, but still uncommon, with formulations based on the semi-permanent fillers poly-L-lactic acid or calcium hydroxyapatite (six events each; 0.7% and 2.6% of procedures, respectively). An additional advantage of non-permanent filler materials is that any complications are likely to be less persistent and easier to treat.[41] Severe complications are extremely rare with these procedures.[38–41] In a review of over 4,500,000 procedures (using formulations based on non-permanent, semi-permanent or permanent filler materials) over a 2-year period from 2010 to 2011 in the United States, only five severe complications were reported (0.0001% of procedures).[41]

Very few adverse hypersensitivity reactions have been reported after injection of hyaluronic acid-based filler formulations.[39] As this compound has no organ or species specificity, there is a low theoretical risk of allergic reaction.[39] Reported outbreaks of cutaneous mycobacterial infections associated with various cosmetic procedures, including mesotherapy, appear to be related to inappropriate cleansing of equipment with contaminated tap water or other sources, rather than contamination of the injection solution itself.[42]

Evidence supporting the use of polycomponent mesotherapy

In spite of the rationale behind the use of mesotherapies as skin anti-aging treatments, rigorous scientific evidence regarding therapeutic efficacy from clinical trials remains relatively scarce in the public domain, although these treatments have been used extensively in practice for many years.[36,37,43–48] Two small clinical studies (n=5–10) of mesotherapy using multivitamin and hyaluronic acid solutions found no significant clinical or histologic changes over 3 to 6 months.[43,47] However, in a recent clinical study involving 55 women with signs of skin aging, a non-cross-linked hyaluronic acid-based mesotherapy formulation with mannitol significantly improved skin elastic parameters and complexion radiance versus control therapy over 3 months.[48] Similarly, a recent study in 50 women who utilized two different mesotherapy formulations (one with hyaluronic acid, vitamins, amino acids, minerals, coenzymes, and antioxidant substances; and a second with hyaluronic acid and the antioxidant idebenone) reported that both formulations provided significant improvements in the clinical appearance of the skin.[49] Another recent study used a combination of both a cross-linked hyaluronic acid filler and a complex non-cross-linked hyaluronic acid-based formulation (with vitamins, antioxidants, amino acids, and minerals) for facial rejuvenation targeting moderate-to-severe wrinkles affecting the nasolabial folds.[50] After 24 weeks, a protocol using both formulations provided significant improvements in skin hydration, transepithelial water loss, and wrinkle esthetic appearance compared with the hyaluronic acid filler alone. A recent long-term, placebo-controlled study, in which mesotherapy with a hyaluronic acid-based formulation was administered to the dorsum of the hand, used high frequency ultrasound to evaluate sub-epidermal low-echogenic band echogenicity as a measure of age-related dermal changes.[37] After treatment for 4 weeks (every week) there was a significant increase in sub-epidermal low-echogenic band versus placebo, indicative of a reduction in photoaging, and this effect was maintained with subsequent monthly treatment for an additional 4 or 10 months.

For other complex polycomponent mesotherapies, no clinical trial data have been published, although topline data for NCTF based on 40 subjects suggest improvements in skin glow, hydration, wrinkles and lines, and cutaneous tonicity with five sessions of therapy with 15 day intervals, along with high levels of patient satisfaction.[25] This is supported by preclinical in vitro and in vivo data evaluating the effects of NCTF on cell proliferation and the synthesis of extracellular matrix, and its effects on antiradical activity (Filorga Laboratories, data on file, 2011).[25,51] In short, NCTF stimulated cell multiplication by 147% versus the untreated control ($P<0.01$) for normal fibroblasts and 148% versus the untreated control ($P<0.01$) for older fibroblasts. The NCTF solution also stimulated intracellular and extracellular collagen synthesis for the normal fibroblasts by 165% and 200%, respectively, versus untreated control (both $P<0.01$). For older fibroblasts, the increases were 166% and 256%, respectively, versus untreated control (both $P<0.01$). Furthermore, NCTF protected human lymphoid (Jurkat) cells from oxidative stress induced by exposure to ultraviolet A (UVA) and UVB radiation, as indicated by a 72% reduction in the level of intracellular hydrogen peroxide ($P<0.01$), and a 90% reduction in intracellular lipid peroxides ($P<0.01$).

Conclusion

Polycomponent mesotherapy solutions, such as NCTF, have been widely and successfully employed for many years as treatments for skin aging. Although the composition of these formulations may appear daunting at first, a consideration of the physiological effects of the individual components can help to demystify these complex therapies. Ultimately, the simple goal of these complex therapies is to create a favorable microenvironment for more optimal fibroblast biosynthetic activity. Preclinical and some clinical trial evidence is available to support the effectiveness of these mesotherapies, as well as long-term international experience in the clinical use of NCTF to deal with a wide range of esthetical and dermatological problems. However, there is a clear need for large-scale, well-controlled clinical efficacy and safety studies in this field.

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Disclosure

The author reports no conflicts of interest in this work.

References

1. Gilchrist B, Krutman J. *Skin Aging*. New York: Springer; 2006.
2. Farage MA, Miller KW, Maibach HI. *Textbook of Aging Skin*. New York: Springer; 2010.
3. Farage MA, Miller KW, Elsner P, Maibach HI. Characteristics of the aging skin. *Adv Wound Care (New Rochelle)*. 2013;2(1):5–10.

4. Friedman M, Friedman B. *Cell Communication: Understanding How Information Is Stored and Used in Cells*. 1st ed. New York: Rosen Publishing Group; 2005.
5. Bradshaw RA, Dennis EA. *Handbook of Cell Signaling*. 2nd ed. San Diego, CA: Academic Press; 2009.
6. Tandara AA, Mustoe TA. MMP- and TIMP-secretion by human cutaneous keratinocytes and fibroblasts – impact of coculture and hydration. *J Plast Reconstr Aesthet Surg*. 2011;64(1):108–116.
7. Salomon D, Saurat JH, Meda P. Cell-to-cell communication within intact human skin. *J Clin Invest*. 1988;82(1):248–254.
8. Tosti A, De Padova MP. *Atlas of Mesotherapy in Skin Rejuvenation*. London: Informa; 2007.
9. Farage MA, Miller KW, Elsner P, Maibach HI. Intrinsic and extrinsic factors in skin ageing: a review. *Int J Cosmet Sci*. 2008;30(2): 87–95.
10. Varani J. Fibroblast aging: intrinsic and extrinsic factors. *Drug Discov Today Ther Strateg*. 2010;7(3–4):65–70.
11. Fisher GJ, Varani J, Voorhees JJ. Looking older: fibroblast collapse and therapeutic implications. *Arch Dermatol*. 2008;144(5):666–672.
12. Fisher GJ, Quan T, Purohit T, et al. Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *Am J Pathol*. 2009;174(1):101–114.
13. Humbert P, Viennet C, Legagneux K, Grandmottet F, Robin S, Muret P. In the shadow of the wrinkle: experimental models. *J Cosmet Dermatol*. 2012;11(1):79–83.
14. Humbert P, Viennet C, Legagneux K, et al. In the shadow of the wrinkle: theories. *J Cosmet Dermatol*. 2012;11(1):72–78.
15. Vedrenne N, Coulomb B, Danigo A, Bonté F, Desmoulière A. The complex dialogue between (myo)fibroblasts and the extracellular matrix during skin repair processes and ageing. *Pathol Biol (Paris)*. 2012;60(1): 20–27.
16. Quan T, Wang F, Shao Y, et al. Enhancing structural support of the dermal microenvironment activates fibroblasts, endothelial cells, and keratinocytes in aged human skin in vivo. *J Invest Dermatol*. 2013;133(3):658–667.
17. Polefka TG, Meyer TA, Agin PP, Bianchini RJ. Cutaneous oxidative stress. *J Cosmet Dermatol*. 2012;11(1):55–64.
18. Tigges J, Krutmann J, Fritsche E, et al. The hallmarks of fibroblast ageing. *Mech Ageing Dev*. 2014;138:26–44.
19. Imokawa G. Mechanism of UVB-induced wrinkling of the skin: paracrine cytokine linkage between keratinocytes and fibroblasts leading to the stimulation of elastase. *J Investig Dermatol Symp Proc*. 2009;14(1):36–43.
20. Nakajima H, Ezaki Y, Nagai T, Yoshioka R, Imokawa G. Epithelial-mesenchymal interaction during UVB-induced up-regulation of neutral endopeptidase. *Biochem J*. 2012;443(1):297–305.
21. Borg M, Brincat S, Camilleri G, Schembri-Wismayer P, Brincat M, Calleja-Agius J. The role of cytokines in skin aging. *Climacteric*. 2013;16(5):514–521.
22. Iorizzo M, De Padova MP, Tosti A. Biorejuvenation: theory and practice. *Clin Dermatol*. 2008;26(2):177–181.
23. Kilberg MS, Häussinger D. *Mammalian Amino Acid Transport: Mechanism and Control*. New York: Springer; 1992.
24. Krutmann J, Schroeder P. Role of mitochondria in photoaging of human skin: the defective powerhouse model. *J Investig Dermatol Symp Proc.* 2009;14(1):44–49.
25. NCTF135,135HA [product information]. Paris, France: Filorga Laboratories. Available from: http://www.global-esthetic.com/uploads/NTCF135,135HA.pdf. Accessed May 6, 2014.
26. Blake S. *Vitamins and Minerals Demystified*. New York: McGraw-Hill; 2008.
27. Combs GF. *The Vitamins. Fundamental Aspects in Nutrition and Health*. 4th ed. London: Academic Press; 2012.
28. Scholz RW, Graham KS, Gumpricht E, Reddy CC. Mechanism of interaction of vitamin E and glutathione in the protection against membrane lipid peroxidation. *Ann NY Acad Sci*. 1989;570:514–517.
29. Noctor G, Foyer CH. Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol*. 1998;49: 249–279.
30. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. *Biochem Pharmacol*. 2003;66(8):1499–1503.
31. Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. *Cell Biochem Funct*. 2004;22(6):343–352.
32. Baumann L. Skin ageing and its treatment. *J Pathol*. 2007;211(2): 241–251.
33. Girish KS, Kemparaju K. The magic glue hyaluronan and its eraser hyaluronidase: a biological overview. *Life Sci*. 2007;80(21): 1921–1943.
34. Ardizzoni A, Neglia RG, Baschieri MC, et al. Influence of hyaluronic acid on bacterial and fungal species, including clinically relevant opportunistic pathogens. *J Mater Sci Mater Med*. 2011;22(10): 2329–2338.
35. Jäger C, Brenner C, Habicht J, Wallich R. Bioactive reagents used in mesotherapy for skin rejuvenation in vivo induce diverse physiological processes in human skin fibroblasts in vitro- a pilot study. *Exp Dermatol*. 2012;21(1):72–75.
36. Lacarrubba F, Tedeschi A, Nardone B, Micali G. Mesotherapy for skin rejuvenation: assessment of the subepidermal low-echogenic band by ultrasound evaluation with cross-sectional B-mode scanning. *Dermatol Ther*. 2008;21 Suppl 3:S1–S5.
37. Tedeschi A, Lacarrubba F, Micali G. Mesotherapy with an intradermal hyaluronic acid formulation for skin rejuvenation: an intrapatient, placebo-controlled, long-term trial using high-frequency ultrasound. *Aesthetic Plast Surg*. Epub December 25, 2014.
38. Alam M, Dover JS. Management of complications and sequelae with temporary injectable fillers. *Plast Reconstr Surg*. 2007; 120(6 Suppl):98S–105S.
39. Requena L, Requena C, Christensen L, Zimmermann US, Kutzner H, Cerroni L. Adverse reactions to injectable soft tissue fillers. *J Am Acad Dermatol*. 2011;64(1):1–34.
40. Daines SM, Williams EF. Complications associated with injectable soft-tissue fillers: a 5-year retrospective review. *JAMA Facial Plast Surg*. 2013;15(3):226–231.
41. Ozturk CN, Li Y, Tung R, Parker L, Piliang MP, Zins JE. Complications following injection of soft-tissue fillers. *Aesthet Surg J*. 2013;33(6): 862–877.
42. Wong SS, Wong SC, Yuen KY. Infections associated with body modification. *J Formos Med Assoc*. 2012;111(12):667–681.
43. Amin SP, Phelps RG, Goldberg DJ. Mesotherapy for facial skin rejuvenation: a clinical, histologic, and electron microscopic evaluation. *Dermatol Surg*. 2006;32(12):1467–1472.
44. Atiyeh BS, Ibrahim AE, Dibo SA. Cosmetic mesotherapy: between scientific evidence, science fiction, and lucrative business. *Aesthetic Plast Surg*. 2008;32(6):842–849.
45. Sivagnanam G. Mesotherapy – The French connection. *J Pharmacol Pharmacother*. 2010;1(1):4–8.
46. Herreros FO, Moraes AM, Velho PE. Mesotherapy: a bibliographical review. *An Bras Dermatol*. 2011;86(1):96–101.
47. El-Domyati M, El-Ammawi TS, Moawad O, et al. Efficacy of mesotherapy in facial rejuvenation: a histological and immunohistochemical evaluation. *Int J Dermatol*. 2012;51(8):913–919.
48. Baspeyras M, Rouvrais C, Liégard L, et al. Clinical and biometrological efficacy of a hyaluronic acid-based mesotherapy product: a randomised controlled study. *Arch Dermatol Res*. 2013;305(8):673–682.
49. Savoia A, Landi S, Baldi A. A new minimally invasive mesotherapy technique for facial rejuvenation. *Dermatol Ther (Heidelb)*. 2013;3(1): 83–93.
50. Iannitti T, Morales-Medina JC, Coacci A, Palmieri B. Experimental and clinical efficacy of two hyaluronic acid-based compounds of different cross-linkage and composition in the rejuvenation of the skin. *Pharm Res*. Epub June 25, 2014.
51. Knoll B. Anwendungsbeobachtung zweier hyaluronhaltiger Mesotherapie-Produkte: REDENSITY I (Teoxane) vs. NCTF HA 135 (Filorga) [Application study of two mesotherapy products containing hyaluronic acid: Redensity I (Teoxane) vs NCTF HA 135 (Filorga)]. *Kosmet Med*. 2012;33:60–63. German.