INJECTIONS OF COMBINATIONAL ACTIVES TO REJUVENATE FACIAL SKIN

Sabine Zenker, Enrique Lorente, Fernando Galcerán, Rafaela Vidal, Ignacio Ordiz, Josefina Martinez, and **Luis Luis** discuss using injections of hyaluronic acid, polynucleotides, organic silicon, and DMAE to rejuvenate the skin

ABSTRACT
Healthy and beautiful skin features are one of the first aesthetic eye catchers. Typical signs of ageing facial skin are the loss of its hydration and firmness as well as skin sagging with the appearance of wrinkles, creases and folds. This study was completed to evaluate safety and efficacy of various, partly innovative, mixtures of poly-active agents injected into the ageing facial skin in a mesotherapy mode such as hyaluronic acid, polynucleotides, organic silicon, and dimethylethanolamine to rejuvenate facial skin.

SKIN AGEING IS AN ONGOING process, typically dividable into two mechanisms: extrinsic and intrinsic ageing. The primary trigger for skin changes from extrinsic factors is photoaging. The pathomechanism is the formation of free radicals causing oxidative damage as well as the induction of an inflammatory milieu taking place in both the epidermis and dermis. Overexpression of metalloproteinases (MMP) speeds up the ageing process through collagen degradation. The complex process results in a decrease in collagen type I and degeneration of extracellular fibres[1], with atrophy in all layers, thinning of the epidermis and dermis with reduced elasticity, the formation of wrinkles and folds (solar elastosis) as well as irregular pigmentation, broken vessels, and possibly skin cancer and its precursors.

Intrinsic skin ageing is a process based on chronobiologic, genetically determined ageing influenced by hormones, such as oestrogen, responsible for skin elasticity, water retention, and circulation; androgens, which increase sebum production; and gestagens, which inhibit the enzymatic depletion of connective tissue. The reduction of hormones present in menopause results in typical skin changes, such as the reduced thickness of the epidermis, reduced proliferation activity of the keratinocytes, and a diminished capacity for differentiation. Throughout the dermis, a depletion of up to 30% of collagen fibres occurs in the first 5 years of menopause and skin matrix proteins are also reduced. The skin gets rough, wrinkly, more sensitive, more easily vulnerable, and benign lesions such as seborrheic keratosis can occur.

So, summarizing the structural and functional changes occurring at the epidermis and dermis during the ageing process, one can see again alterations in dermal collagen, elastin and glycosaminoglycans[1]; a loss in the content of hyaluronic acid resulting in skin dehydration and loss of turgidity with overall thinning; and loss of elasticity and impaired response to ultraviolet light[2,3].

Mesotherapy

Mesotherapy to treat aged skin works through multiple micro-dosed superficial injections using specific injection techniques, such as epidermal-nappage, micro-papular or the point-by-point technique to inject bioactive substances in the skin to treat cosmetic conditions[4]. The therapeutic aim of mesotherapy for skin rejuvenation is to increase the activity of the fibroblasts by inducing an optimal physiologic environment to enhance the cell activity and, therefore, encourage the synthesis of collagen, elastin, and hyaluronic acid. ▷

SABINE ZENKER, MD
Dermatologist
DrZenkerDermatology
Maximilianstraße 16
D-80539 Munich, Germany

email: kontakt@dr-zenker-services.de

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KEYWORDS
Ageing skin, mesotherapy, hyaluronic acid, polynucleotides, organic silicon, dimethylethanolamine

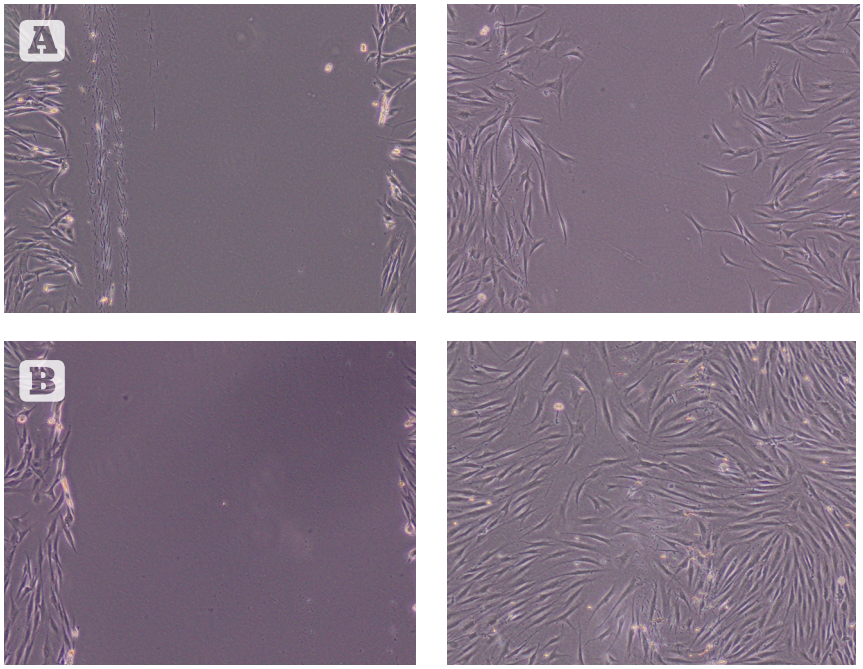

Figure 1 *In vitro* study fibroblasts/polynucleotides. Twenty-four hours after treatment with HA, normal dermal cell regeneration capacity increases by 96%. Assessment of dermal regeneration capacity (fibroblast proliferation and migration) in base culture medium (Control) (A) or base culture medium supplemented with HA (B). Images captured at 0h and after 24h with the optical microscope. Source: Biotechnology Unit. mesoestetic Pharma Group S.L. 2016. Product used: mesohyal™ Hyaluronic

▷ The desired clinical result is firm, bright, and moisturised skin. Typically, a liquid mixture of compounds such as hyaluronic acid, pharmaceutical and homoeopathic medications, plant extracts, vitamins and other ingredients, such as platelet-rich plasma is used to improve the overall quality of the skin. Hyaluronic acid especially has proven to increase the skin's elasticity, hydration, and firmness[4].

In vitro data

It is well known that injection of non-reticulated hyaluronic acid (HA) attracts water molecules, therefore hydrating the tissue and stimulating collagen synthesis. Hyaluronic acid is also involved in other processes, such as wound healing, neutralisation of free radicals, and it interacts with proteoglycans in the extracellular matrix[5,6].

Dimethylethanolamine (DMAE) is a precursor of acetylcholine, a neurotransmitter that intervenes in muscle contraction. It increases epidermal and dermal thickness, enhances stratum corneum water content and inhibits the formation of lipofuscin (pigment)[7-12].

Recent studies show that mesotherapy by injecting DMAE combined with amino acids (AA) can regulate the collagen catabolism resulting in collagen stimulation[13]. Other studies show a decrease in the production of collagen type I and an increase of collagen type III and MMP-1 in ageing skin treated likewise[14]. Results obtained from these studies indicate that mRNA expression for collagen type III and MMP-1 is much less in ageing cells than that of normal tissue[14]. It might be that collagen metabolism undergoes a different process between regular skin changes with ageing and a D-galactose-induced subacute skin ageing model[14]. According to this study, injection of DMAE or AA alone showed no effects on hydroxyproline content or messages for type I collagen and MMP-1 in ageing skin. These data indicate that the concomitant use of DMAE and AA might be the only way to exert their antiageing action in this D-gal induced ageing skin model, by modulating collagen type I metabolism and remoulding the structure of the ageing skin[14].

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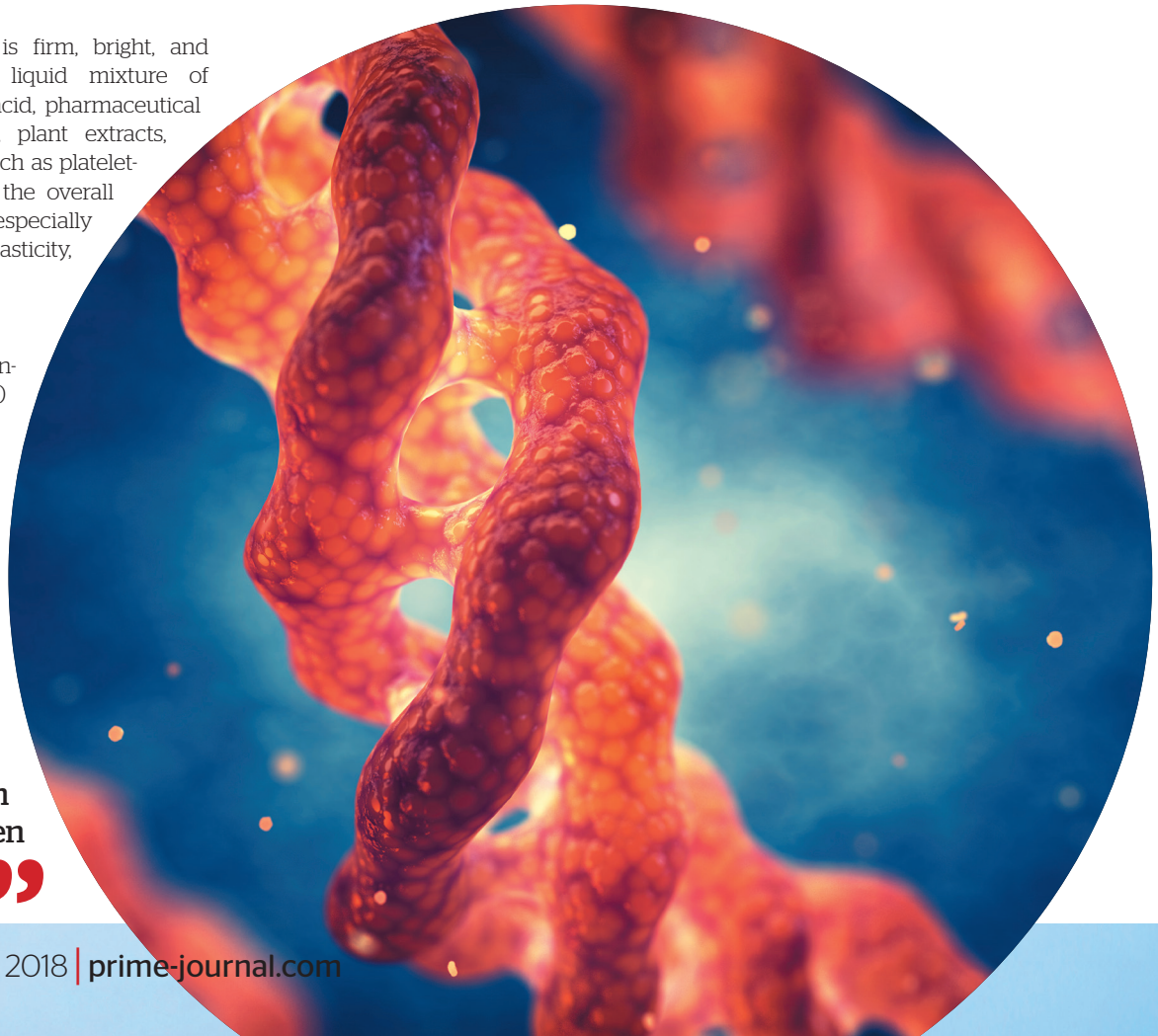

Organic silicon

Organic silicon, an element the skin is rich in, acts at a structural level ensuring the integrity, tone, and elasticity of cutaneous tissue and binds to collagen, elastin, and proteoglycans. The reserves of silicon deplete progressively with age. However, a silicon supplement can improve skin hydration and tone, resulting in firmer, softer skin with a greater repair capacity. Indexed studies report that fibroblast culture irradiated with UV-A rays to simulate photoaging produce less collagen and present less resistance to traction (less elasticity). Thus, organic silicon has the potential to stimulate fibroblasts to synthesise collagen and to increase skin elasticity[15,16].

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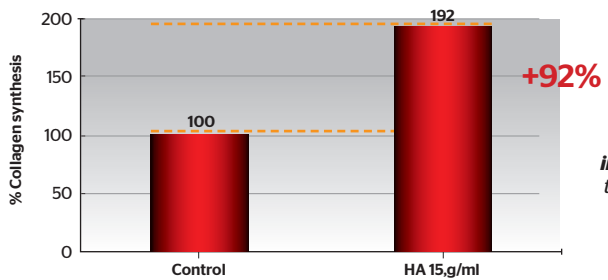
Polynucleotides (PN) are biopolymers composed by nucleotide monomers DNA (deoxyribonucleic acid), and RNA (ribonucleic acid) are examples of PN. Polynucleotides have shown to improve tissue regeneration[17-19]. On the one hand, nucleotides are composed of a nitrogenous base, a pentose sugar and phosphoric acid. On the other hand, polynucleotides are organic compounds formed by covalent bonds between nucleotides.

Figure 2 *In vitro* study fibroblasts/polynucleotides



In vitro collagen trial with human fibroblasts and non-reticulated hyaluronic acid (HA) obtained by bio-fermentation. Assessment of the quantity of collagen produced by human fibroblasts in base culture medium (Control) or base culture medium supplemented with HA. Source: Biotechnology Unit. mesoestetic Pharma Group S.L. 2016. Product used: mesohyal™ Hyaluronic

The diagram shown in *Figures 1-2* highlights the results of an *in vitro* trial to improve cell regeneration of human fibroblasts by adding non-reticulated hyaluronic acid (HA) to the cell culture. Twenty-four hours post treatment the fibroblasts showed an increased dermal cell regeneration capacity by 96%.

Twenty-four hours post-treatment with HA, the fibroblasts showed an increased collagen synthesis capacity by 92%. When polynucleotides were added to the culture medium, an increase in the number of ▷

Figure 3 (A) before (B) after treatment of a 49 year old female patient with 2.5 ml mesohyal™ X-DNA + 2.5 mesohyal™ DMAE

Figure 4 (A) before (B) after treatment of a 44 year old female patient with 2.5 ml mesohyal™ Hyaluronic + 2.5 mesohyal™ Organic Silicon

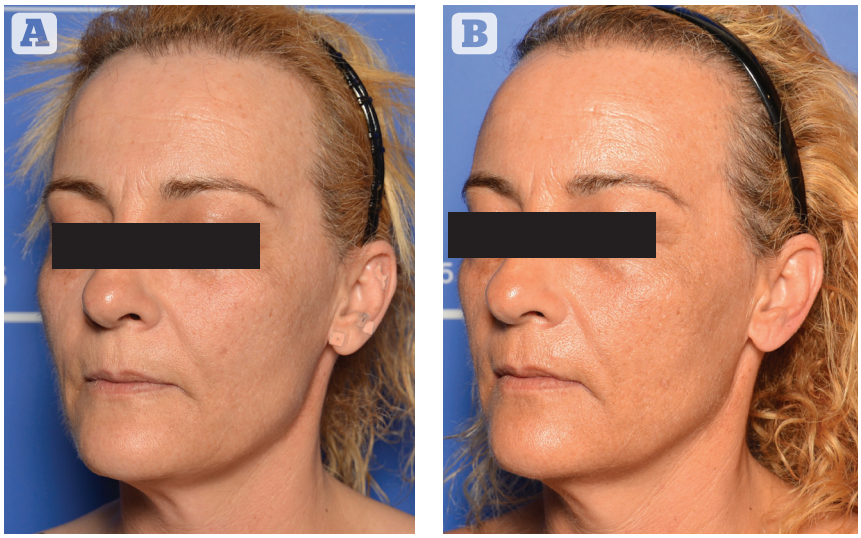


Figure 5 (A) before (B) after treatment of a 49 year old female patient with 2.5 ml mesohyal™ X-DNA + 2.5 mesohyal™ DMAE

▷ fibroblasts and the synthesis of extracellular matrix proteins (collagen, elastin and fibronectin) was recorded.

Other studies proved the synthesis of growth factors (EGF, FGF, etc.)[20] and enhanced cell protection against solar radiation[21].

In vivo data

Material and methods

A prospective study was performed with twenty Caucasian women aged 41 to 69 years, with a Glogau score of 2 or higher. Volunteers were divided into two subgroups: Subgroup A – those experiencing dehydration and loss of elasticity, and Subgroup B – those women experiencing sagging. All subjects received five mesotherapy treatment sessions for facial skin ageing using either organic silicon and sodium hyaluronate (concentration of 2.5mg/ml) or polynucleotides, dimethylaminoethanol, and sodium hyaluronate (2.5 mg/ml), respectively. Injections were performed intradermally. Efficacy was evaluated objectively by the cutometer for elasticity (Subgroup A) and firmness (Subgroup B). The corneometer evaluated hydration

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levels. Safety was assessed for each treatment session.

Subgroup A included individuals with signs of dehydration and loss of elasticity. Their treatment was based on mesohyal™ Hyaluronic + mesohyal™ Organic Silicon.

Subgroup B included individuals who had marked flaccidity. The treatment was based on mesohyal™ X-DNA + mesohyal™ DMAE. Treatment was applied in five sessions at intervals of 15 days between each visit. In both groups the facial administration protocol was the same.

Technique

Mesotherapy-wise intradermal injections to the skin of the face to the epidermis and superficial dermis, approximate injection depth 3 mm, the approximate amount of product is 0.05ml/ppt using a 1-3 ml Luer-lock syringe with a 4 mm 30G sharp needle. The total volume of product injected per session was approximately 3-5 ml/session; 5 treatments in total.

Evaluation of efficacy was completed at two follow-up visits (after 3 sessions and one week after the end of treatment) using a Cutometer™ probe (Courage - Khazaka Electronic) for skin elasticity measurements in Subgroup A and firmness evaluation in Subgroup B. Additionally, ▷

Figure 6 (A) before (B) after treatment of a 51 year old female patient with 2.5 ml mesohyal™ Hyaluronic + 2.5 mesohyal™ Organic Silicon

▷ a self-assessment was performed by each patient after completion of the study.

Safety assessment evaluated both immediately after each session and 15 days after each session with no unexpected side-effects or adverse events reported.

Results

The results in Subgroup A (hyaluronic acid+organic silicon) showed that skin elasticity improved up to 19.4%, while with Subgroup B firmness improved up to 25.4%. In both subgroups, a noticeable improvement was observed in assessing the fine wrinkles and skin hydration in over 85% of the volunteers. As per the self-assessment, 90% of the volunteers reported a noticeable improvement in the hydration of their facial skin after five sessions.

In Subgroup B (polynucleotides+DMAE+sodium hyaluronate) 80% of the volunteers reported a noticeable improvement in skin hydration after 5 months of treatment.

The intradermal administration of mesohyal™ X-DNA and mesohyal™ DMAE showed an improvement in skin elasticity of 19.35% after five sessions of treatment, according to objective data obtained from the Cutometer™.

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Key points

- Mesotherapy is an effective therapeutic tool to improve skin quality
- Amongst the large variety of injectables, the combination of organic silicone/polynucleotides/ hyaluronic acid and DMAE have scientifically prooven to increase elasticity, irmness and hydration of the skin

Conclusion

Typical signs of skin ageing are dehydration and its loss of elasticity and firmness. This study data evaluates changes in skin elasticity, firmness, and hydration after intradermal injection of different actives such as non-reticulated hyaluronic acid, polynucleotides, organic silicon, and dimethylethanolamine to the facial skin as well as the treatment's safety and efficacy. Both combined mesotherapy protocols proved to be an effective and safe tool to improve the overall skin quality suited to treat ageing facial skin.

▶ ***Declaration of interest*** *None*

References

1. Fisher, G et al. Looking older: Fibroblast Collapse and Therapeutic Implications. Arch Dermatol. 2008 May ; 144(5): 666–672
2. Fenske NA et al. Structural and functional changes of normal aging skin. J Am Acad Dermatol. 1986 Oct;15(4 Pt 1):571-85
3. Coleman SR et al. The anatomy of the aging face: volume loss and changes in 3-dimensional topography. Aesthet Surg J. 2006;26(suppl 1):S4-S9. Zenker, S. Combination Therapies: From Surface to Shape. Anti Age Magazine 18, 2015; 110-111
4. Herreros FO. Mesotherapy: a bibliographical review. An Bras Dermatol 2011 Jan-Feb; 86(1):96-101
5. Baspeyras M et al. Clinical and biometrological efficacy of a hyaluronic acid-based mesotherapy product: a randomised controlled study. Arch Dermatol Res. 2013 Oct;305(8):673-82
6. Reuther T et al.. Effects of a three-session skin rejuvenation treatment using stabilized hyaluronic acid-based gel of non-animal origin on skin elasticity: a pilot study. Arch Dermatol Res. 2010;302:37-45
7. Tadini KA et al. In vivo skin effects of a dimethylaminoethanol (DMAE) based formulation. Pharmazie. 2009 Dec;64(12):818-22
8. Uhoda I et al. Split estudio sobre el efecto de la tracción cutánea de 2-dimetilaminoetanol (deanol) gel. Skin Res Technol 2002 Aug; 8 (3) :164-7
9. Londres ED et al. Aumento farmacológico de los niveles de acetilcolina en el estriado de rata kainato lesionado. Biochem Pharmacol 1978, 27: 2962-2965
10. Haubrich DR et al. Aumento de la acetilcolina de cerebro de rata inducida por la colina o deanol. Life Sci. 1975, 17: 975-980
11. Jope RS et al. Dimetilaminoetanol (deanol) el metabolismo en el cerebro de rata y su efecto sobre la síntesis de la acetilcolina. J Pharmacol Exp Ther 1979, 211:472-479
12. Zs.-Nagy I. intervenciones farmacológicas contra el envejecimiento a través de la membrana plasmática de la célula, una revisión de los resultados experimentales obtenidos en animales y humanos, Anales de la Academia de Ciencias de Nueva York de 2002, 959:308-320
13. Su, L. Zhenyu, C. Xia, Cai, Y. et al. "Effects of Dimethylaminoethanol and Compound Amino Acid on D-Galactose Induced Skin Aging Model of Rat," The Scientific World Journal, vol. 2014, Art. ID 507351. 2014
14. J. Liang, X. Pei, Z. Zhang et al., "The protective effects of long-term oral administration of marine hydrolysate from chum salmon on in the chronological aging Sprague-Dawley male rats," Journal of Food Science, vol. 75, no. 8, pp. H230-H238, 2010
15. Robin S et al. International Journal of Cosmetic Science, 2012, 34, 311-317
16. Petersen Vitello Kalil CL et al. Evaluation of cutaneous rejuvenation associated with the use of ortho-silicic acid stabilized by hydrolyzed marine collagen. J Cosmet Dermatol. 2017 Sep 20. doi: 10.1111/jocd.12430. [Epub ahead of print]
17. Park KY et al. Long-chain polynucleotide filler for skin rejuvenation: efficacy and complications in five patients. Dermatol Ther. 2016 Jan-Feb;29(1):37-40. doi: 10.1111/dth.12299. Epub 2015 Nov 2
18. Robin S et al. International Journal of Cosmetic Science, 2012, 34, 311-317
19. Petersen Vitello Kalil CL et al. Evaluation of cutaneous rejuvenation associated with the use of ortho-silicic acid stabilized by hydrolyzed marine collagen. J Cosmet Dermatol. 2017 Sep 20. doi: 10.1111/jocd.12430. [Epub ahead of print]
20. Chavan, A. Biochemestry, 33, 7193-7202 (1994)
21. Musk, P et al. Mutat. Red., 227, 25-30 (1990)
22. Jenkins G. Mech agening Dev. 2002, 123 (7): 801-10
23. Naylor EC. Maturitas. 2011; 69 (3): 249-56
24. Rinnerthaler M. Biomolecules. 2015; 5 (2):545-89
25. Guyuron B. Plast Reconstr surg. 2009; 123 (4): 1321-31
26. Rinnerthaler M. Biomolecules. 2015;5 (2):545-89
27. Doshi DN. Arch Dermatol.2007; 143 (12):1543-6
28. Raschke GF. J Craniomaxillofac surg 2014. 42 (5):e312-7
29. Ichibori R. J Cosmet Dermatol. 2014; 13 (2): 158-63
30. B. Sommerfeld, "Randomised, placebo-controlled, doubleblind, split-face study on the clinical efficacy of Tricutan on skin firmness," Phytomedicine, vol. 14, no. 11, pp. 711–715, 2007
31. Uhoda, N. Faska, C. Robert, G. Cauwenbergh, and G. E. Pi´erard, "Split face study on the cutaneous tensile effect of 2-dimethylaminoethanol (deanol) gel," Skin Research and Technology, vol. 8, no. 3, pp. 164–167, 2002