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AMINO ACID AND HYALURONIC ACID MIXTURES DIFFERENTIALLY REGULATE EXTRACELLULAR MATRIX GENES IN CULTURED HUMAN FIBROBLASTS

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The aim of this screening study was to evaluate the efficacy of different proprietary mixtures of amino acid and hyaluronic acid (HA) in stimulating the production of extracellular matrix (ECM) components, particularly the neo-synthesis of elastin, and in promoting a more efficient deposition of elastic fibres (elastogenesis), while at the same time maintaining the stimulation of collagen. The study has allowed identification of the optimal ratios between the amino acids (AA) for the production of collagen and elastin. Human primary dermal fibroblasts from a 44-year-old female donor were used as a test system in an experimental design based on the evaluation of the expression of relevant ECM genes using a transcriptomic dynamic approach. The expression of ECM genes was evaluated by RTqPCR from 24 to 120 hours in the presence of the test items. Moreover, the production of ECM proteins was verified by Western blot analysis after a 120 h treatment period. In addition to elastin, collagen IV, a fundamental structural component of the basal lamina responsible for epithelial and connective tissue anchoring, was analysed as potential target for the modulation of ECM protein production by human fibroblast. The first phase of the study demonstrated that alanine and valine are essential to promote production of elastin, of which they are important constituents. The second phase of the study, which was conducted to clarify the interactions between the different clusters of AA, demonstrated that it is necessary to choose a mixture that contains specific amounts of amino acids of both proteins, collagen and elastin, to give a significant response and a significant production of both. This also proves the existence of a ratio between the 2 clusters (AA elastin/AA collagen) that guarantees an adequate and balanced response to gene expression and production by fibroblasts, collagen and elastin. The study has allowed identification of the optimal ratios between the AA for the production of collagen and elastin.

The use of biomaterials, a broad category of materials that also comprises biopolymers for clinical application, is becoming increasingly important and hyaluronic acid (HA), in particular, is one of the most used. Due to its unique physical characteristics (hygroscopicity and visco-elasticity), it is largely employed in several fields of medicine including: ophthalmology, dentistry, rheumatology and regenerative and aesthetic medicine (1-5). In addition to its mechanical and physical properties, HA has important biological functions, binding specific membrane receptors (6), regulating cellular adhesion, proliferation and migration, cytokine synthesis and deposition of extra cellular matrix (ECM) proteins (7-8).

It has been demonstrated that adding small molecules, such as mixtures of amino acids (AA) (e.g. proline, lysine, glycine and leucine), to HA can enhance its action in many fundamental biological processes, and mainly in wound healing (9-10).

Key words: amino acids, fibroblasts, elastogenesis, elastin, collagen

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The biological wound healing process is one the most complicated processes described in relation to the skin and involves different and overlapping phases such as inflammation, proliferation, migration of keratinocytes, new tissue formation and tissue remodelling: all these steps are necessary to achieve tissue regeneration and wound healing (9).

All these phases are characterized by biochemical, molecular and morphological changes and even if not entirely characterized, they can be monitored in the different skin compartments thanks to identification of the relevant bio-markers. During the latter phases (re-modelling), ECM proteins, which are massively synthetized, assembled and reorganized by fibroblasts allowing tissue reconstruction, play a critical role (11).

These proteins include collagens and fibronectin, the biosynthesis of which is strictly connected to the local bioavailability of constituent AA. Collagen triple helix consists of glycine, L-proline and L-lysine in well-determined positions. Following the activation of specific hydroxylases (e.g. LOXL1) by L-lysine, the hydroxylation of glycine and proline residues causes the formation of inter- and intra-molecular bonds, which give collagen its viscoelastic properties (12). It has been shown that the use of an AA-enriched HA (Aminogam®) promotes the expression of type I and III collagen, the major components of connective and granulation tissues respectively, and of fibronectin genes in human pulmonary fibroblasts (13). Moreover, the expression of ECM deposition regulators [such as TGFβ and, connective tissue growth factor (CTGF)] has been shown to be increased by the treatment (13).

Recently, attention has been paid to other components of ECM in order to investigate the further effects of HA mixtures and to discover other potential clinical applications (14). Of the components of ECM, elastic fibres play a fundamental role for the structure and function of elastic tissues such as the bladder, cartilage, ligaments, blood vessels and the skin.

The elastic fibres consist of microfibrils and elastin. Microfibrils mainly consist of fibrillin, and provide a scaffold which guides the alignment and the cross-linking of elastin molecules by one or more members of the lysyl oxidase (LOX) gene family (15).

Elastin is a polymeric protein derived from the

Table I. *Amino acid composition of the tested formulations.*

		%			
		Mixture A	**Mixture B**	**Mixture C**	**Mixture D**
COLLAGEN	Glycine	50	34.8	33.4	30.2
	L-Proline	37.5	26.1	25.1	22.7
	L-Lysine HCl	5.5	3.8	3.7	3.3
ELASTIN	L-Leucine	7	4.9	8.7	4.2
	L-Valine	-	12.9	12.3	16.8
	L-Alanine	-	17.5	16.8	22.8

Table II. *TaqMan probes for genes of interest and endogenous control used in RTqPCR analysis.*

Gene	Code	Taqmas assay no.
Elastin	ELN	Hs00355783_m1
Collagen IV	COL4A1	Hs00266237_m1