COMPARISON OF THE BIOMECHANICAL AND BIOSYNTHETIC BEHAVIOR OF NORMAL HUMAN FIBROBLASTS AND FIBROBLASTS ISSUE FROM A FOREHEAD WRINKLE

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Summary

The wrinkles correspond to the most obvious expression of skin ageing and are manifested by changes on the organization and dermal structure. In the extracellular matrix, decreased quantities of collagens and glycosaminoglycans as well as a deterioration of the fibrillary network is noted, result in a reduction of dermal thickness. In addition, the activity of the collagenases increases in contrast to the synthesis of collagen fibers.

Nor are cells spared during the aging process. We thus studied and compared the contractile capacity as well as the synthesis capacity of normal human fibroblasts and human fibroblasts obtained from biopsies of forehead wrinkles. The capacity of the fibroblasts to be adhered to the collagen network and to maintain a three-dimensional structure of dermis was studied on a model of equivalent dermis. The metabolic activity was studied by evaluating the capacities of synthesis of collagen I, main component of dermis.

Human fibroblasts resulting from the forehead wrinkle contract less the gel of collagen than the normal human fibroblasts and present an activity of biosynthesis of collagen I less important than normal human fibroblasts.

These results show that fibroblasts with aging present a deceleration of their metabolic activity and lose their capacity of adhesion to collagen fibers thus limiting the possibility of organizing the dermal tissue.

We investigated the potential of an active ingredient able to compensate for the reduction of the metabolic activity and to restore the contractile capacity of fibroblasts obtained from forehead wrinkles. This effect was compared with a reference molecule: the vitamin C.

Introduction

The principal changes occuring during ageing are manifested as skin that is thinner, dry and less elastic. Wrinkles, which appear on the skin surface, correspond to the expression of cutaneous ageing and result in changes in the macromolecular structure and composition of the dermis [1]. Considerable work during the past decade has led to the characterization in vivo and in vitro of these modifications but the role of the different acting is just starting to become understood.

Wrinkles seem mainly connected to a rigidification of the skin (of both the *stratum corneum* and the dermis) coupled with a slackening of the dermis [2, 3]. This is manifested in the extracellular matrix by decreased quantities of collagens and glycosaminoglycans, which leads to a reduction of dermal thickness (loss of echogenicity) [4, 5, 6]. Fibroblasts are strongly implied in this phenomenon. With the age, their metabolic activity decreases and they lose their capacity for adhesion to collagen fibers thereby limiting the possibility of organizing dermal tissue [7]. Johnson showed that fibroblasts obtained from donors of different age presented a general deceleration in term of protein synthesis [8]. Moreover, fibroblasts aged artificially by successive passages synthesize collagens of the type I and III in quantity more limited than young fibroblasts [9, 10, 11].
To develop 3D computer model of the wrinkle, Green et al. [12] made a histological study of the wrinkles around the eyes. During this work, they could show a marked reduction in the precursors of the collagen fibrils at the base of the wrinkles: procollagenes I and III and they reported alterations of dermal matrix. These results confirmed those already obtained previously [9, 13, 14]. They also showed the key structural role of the arrangement and the disposition of deep collagen fibers at the base of wrinkles.

The behavior of the old fibroblasts was largely studied, however few studies were carried out concerning operation old fibroblasts resulting directly from a wrinkle. One can suppose that this behavior differs what is the case for fibroblasts obtained from skins with striae, which are characterized by a deterioration of the dermal matrix and present mechanical properties different from those of normal fibroblasts. Moreover, fibroblasts of stretch mark contract collagen gels more slowly than normal human fibroblasts [15].

We are thus more particularly interested in the behavior of human fibroblasts obtained from biopsies of forehead wrinkle. The aim of this study was to investigate the biomechanical and biosynthetic behavior of fibroblasts from wrinkles and to compare it with that normal fibroblasts.

To study the synthesis activity of fibroblasts, we chose as marker collagen I, main collagen of the dermal matrix. The study of the biomechanical behavior of fibroblasts from wrinkles was carried out using a three-dimensional model of collagen gels.

We were then interested in the possibility of restoring and of compensating for the losses of capacity of synthesis and organization of a collagen gel by fibroblasts from wrinkles using a plant extract. During this study we showed that selected soy peptides were able to improve the capacities of synthesis and the contractile capacity of fibroblasts from wrinkles.

Methods

• Fibroblast culture

Fibroblasts were obtained using the explant method from skin samples. Fibroblasts were maintained in MEM medium (cat N° 41090, Invitrogen) supplemented with 10% fetal calf serum (FCS), 1% penicillin (5000 U/ml), 1% streptomycin (5000µg/ml) and fungizone-amphotericin B (250µg/ml) at 0.1% (v/v). They were then placed in a 37°C incubator in a humid atmosphere containing 5% CO_2. Fibroblasts cultures were sub cultured by tripsinization and fibroblasts from the 4th to the 8th passages were then assayed for biosynthetic activities using confluent monolayer cultures and three-dimensional cultures in attached collagen gels.

• Preparation of collagen gels

Three-dimensional collagen gels were prepared in 60 mm Petri dishes (cat. N° 1016, Falcon). Collagen type I was obtained from Institut Jacques Boy, France. For incorporation into collagen gels, fibroblasts were harvested from confluent cultures by trypsinization, counted, adjusted to the desired density (750 000 cells/ml). A collagen mixture was prepared by quickly rat-tail collagen type I solution (2 mg/ml) with a neutralization solution (pH 7,4) containing MEM 2X and FCS. Fibroblasts were added to collagen type I solution at a final concentration of $3,5\times10^4$ cells/ml. After 24 hours, the gel is separated. Gel diameters are measured every day during three days. Measures were realized on 3 lattices.

• Measurement of collagen type I synthesis

Fibroblasts are incubated 48 hours. Supernatants are then recovered. The immunostaining of collagen was performed using murine anti-collagen I monoclonal antibody (cat N° CP17L, Oncogene). The second antibody was peroxidase conjugated-murine anti-IgG antibody (cat N° P0447, Dako). Color was developed using a TMB substrate (Abcys).

Results and discussion

• Collagen I synthesis is slowed down with fibroblasts from wrinkles

We studied the capacities of synthesis of fibroblasts obtained from biopsies of forehead wrinkles and normal human fibroblasts.

To determine if the production of collagen of old fibroblasts and normal fibroblasts were different, we quantified the collagen synthesis by the fibroblasts cultivated in monolayer.

Normal fibroblasts relative collagen I rate is higher than this of fibroblasts from wrinkles (figure 1).

When fibroblasts are cultivated in monolayer, fibroblasts from wrinkles have a capacity of collagen I synthesis decreased (-70%) compared to normal fibroblasts.

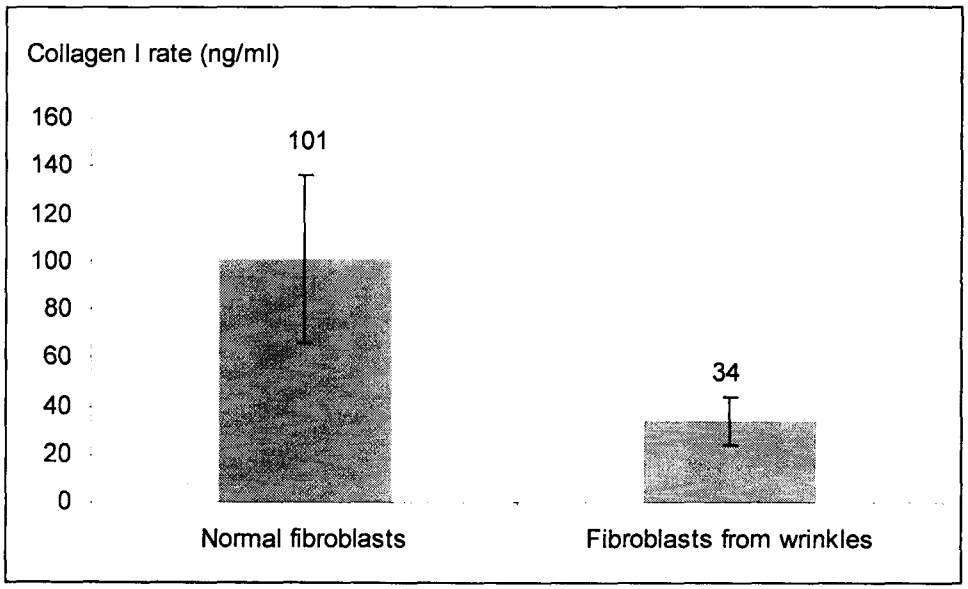


Figure 1: Comparison of the biosynthetic behavior of normal fibroblasts and fibroblasts from wrinkles.

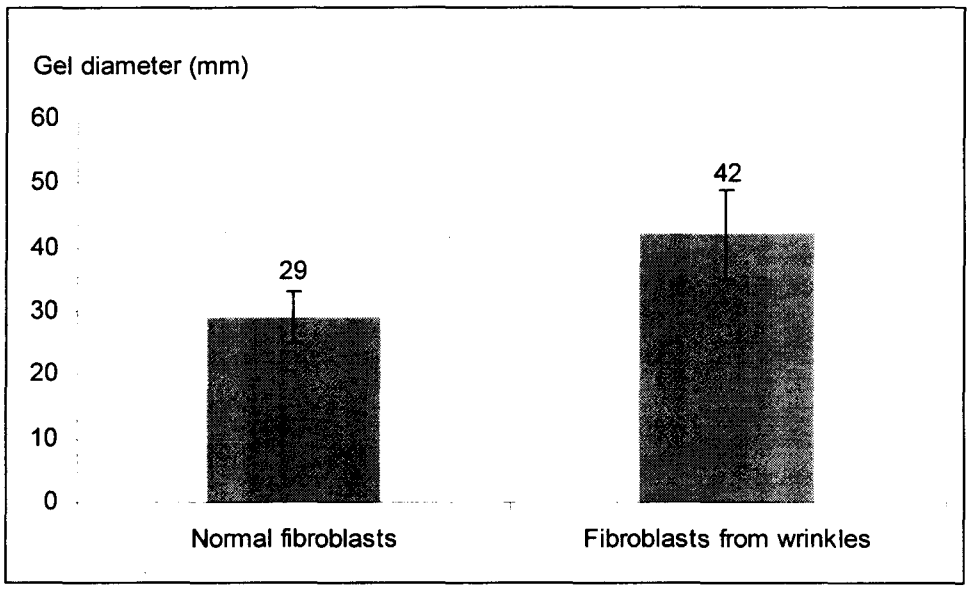
• Contractile capacity of fibroblasts from wrinkles is decreased

The biomechanical behaviour of human fibroblasts from wrinkles and normal fibroblasts was studied while choosing a model of human fibroblasts cultures put in suspension in a collagen gel.

Normal human fibroblasts present important properties of reorganization and reorientation of the collagen fiber [16].

The fibroblasts influence the alignment of collagen fibers, which in their turn affect the distribution of fibroblasts [14]. These properties are manifested physically in the lattices by the contraction of the gel.

This phenomenon is mainly connected to the locomotion of cells inside the collagen lattices [16]. At the time of this study, we showed that normal human fibroblasts put in suspension in a collagen gel have the capacity to contract and by the same one to reorganize collagen fibers in contrast to fibroblasts from wrinkles which have capacities of contraction decreased compared to those of normal cells (figure 2). The contractile capacity of normal fibroblasts is 1,5 times superior with that of fibroblasts from wrinkles.



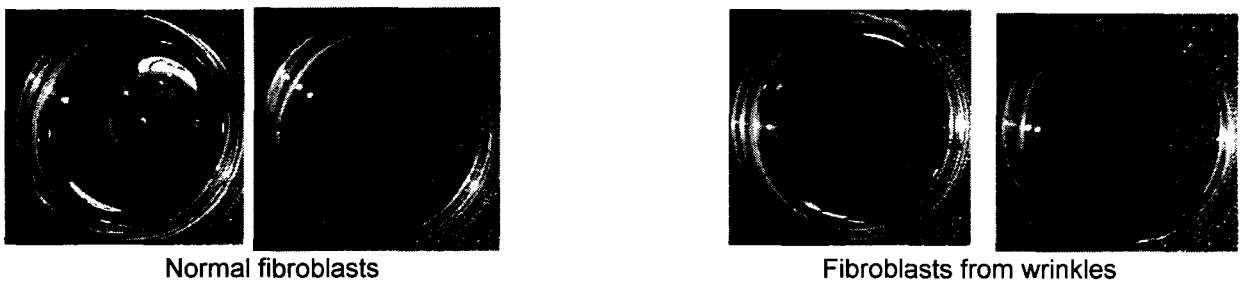


Figure 2: Comparison of the biomechanical behavior of normal fibroblasts compared to the basic fibroblasts of wrinkles after 3 days in a collagen lattices.

• **Study of a vegetable extract able to restore the biomechanical behavior and the capacities of synthesis of basic fibroblasts of wrinkles**

We studied the possibility of restoring the contraction and synthesis capacities of fibroblasts from wrinkles by using the potential of an active ingredient obtained after screening of several raw materials. We found that selected peptides of soy seemed to be most effective to promote the collagen I synthesis and the contractile capacity of human fibroblasts from the base of wrinkles.

Collagen I synthesis

The behavior of the normal fibroblasts and the basic fibroblasts of wrinkles in term of collagen I synthesis was studied in the presence of peptides of soy selected with various concentrations or the vitamin C, reference molecule known to stimulate the metabolism of many dermal proteins [17].

Tested on normal human fibroblasts, the selected soy peptides at 2% for which the rate of vitamin C contained in the extract is of 0,10µg/ml, active the synthesis of collagen I by 175% compared to the control. The vitamin C with 0,10µg/ml tested under the same conditions only supports the collagen synthesis by 158% (figure 3).

Same manner, the synthesis activity of fibroblasts from the base of wrinkles is improved in the presence of selected soy peptides. This effect is higher than that of the vitamin C.

In the presence of selected soy peptides at 2%, the collagen I rate of wrinkles fibroblasts is of 94ng/ml and approaches that of normal human fibroblasts untreated (101ng/ml). The presence of soy peptides in the culture medium of fibroblasts from wrinkles enables them to recover the collagen I basic level of normal human fibroblasts (figure 4).

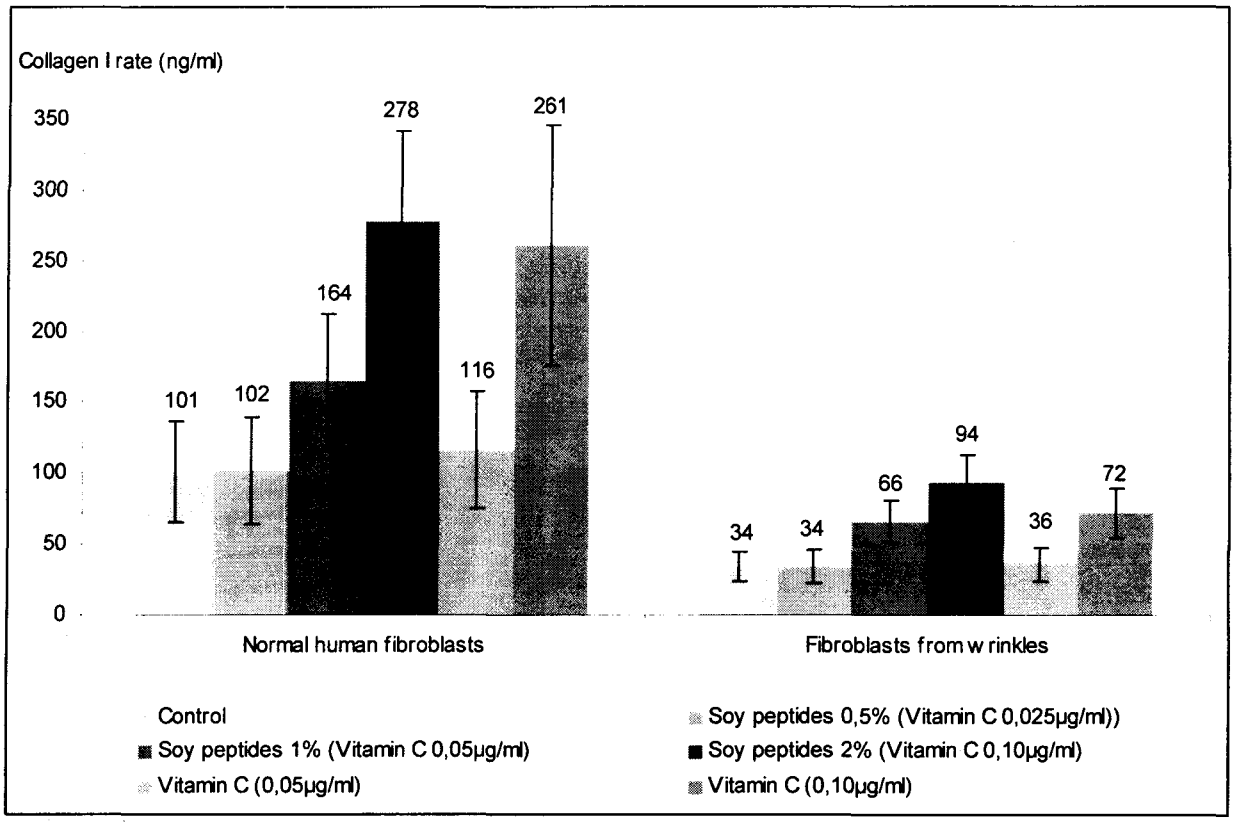


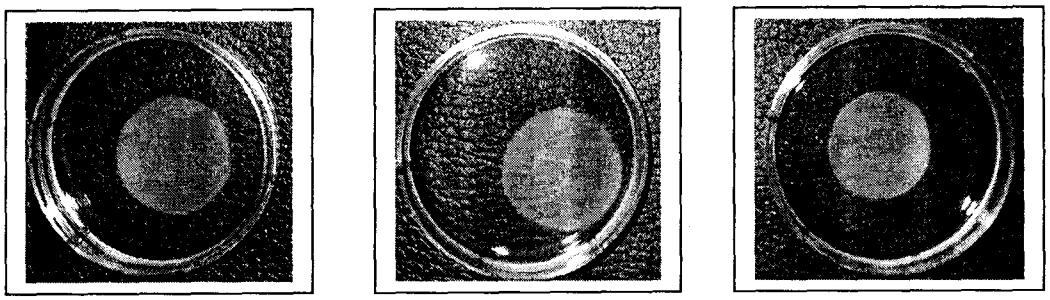
Figure 3: Effect of selected peptides of soy on the synthesis of collagen I of normal human fibroblasts and fibroblasts from the bas of wrinkles.

Collagen gel contraction

As seen previously, fibroblasts from the base of wrinkles present a contractile capacity lower than that of normal fibroblasts. Figure 5 present the effects of selected soy peptides at 2% or the vitamin C at 1µg/ml, reference molecule, on the contraction of collagen gel containing normal human fibroblasts or fibroblasts from wrinkles.

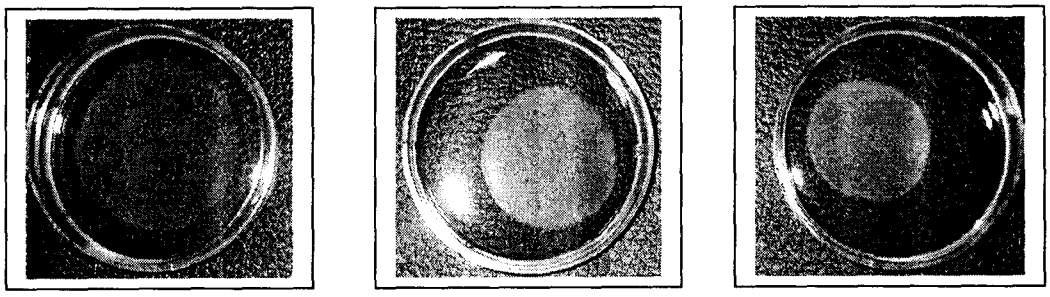
The vitamin C stimulates the contraction of the collagen gel containing normal human fibroblasts in a less great measurement than the selected soy peptides at 2% which slightly supports the contraction of fibroblasts (+3%) compared to the collagen lattices without treatment.

The use of these two molecules in the presence of fibroblasts from the base of wrinkles restores their capacity to contract the collagen gel. In the presence of the selected soy peptides at 2%, the contraction capacity increases by 10% compared to the collagen lattices untreated. The effect of selected peptides of soya was also compared with that of the vitamin C.



Without treatment | Vitamin C 1µg/ml | Selected soy peptides 2%

Normal fibroblasts



Without treatment | Vitamin C 1µg/ml | Selected soy peptides 2%

Fibroblasts from wrinkle

Figure 5: Effect of the vitamin C at 1µg/ml or selected soy peptides at 2% on the contraction of normal fibroblasts and fibroblasts from wrinkles.

CONCLUSION

Our first studies showed the differences in term of biomechanical behavior and capacities of synthesis between normal fibroblasts and fibroblasts from the base of wrinkles. The model of fibroblasts from wrinkles is particularly interesting for better understanding the various modifications, which occur with the age in the cellular metabolism. These changes seem to be one of the keys of the degradation of the mechanical properties of the mature skin, which is manifested on the skin surface by the appearance of wrinkles.

Limiting the appearance of wrinkles thus consists in increasing the synthesis of dermal proteins such as collagen I and III, but also restoring the adhesion and contraction capacities of fibroblasts to the extracellular matrix to thus favor the biomechanical properties of which can again ensure its role of tissue support.

From this report, we tested a certain number of botanical extracts and found that selected soy peptides made it possible to restore at the same time the protein synthesis such as the collagen of type I at fibroblasts from wrinkles and the capacities of reorganization capacities of reorganization of collagen fibers of these cells. This effect could be compared to the vitamin C.

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