Cellular Interconnectivity In Regeneration: A Pioneering Strategy For Healthy Skin

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Abstract (Maximum of 250 words)

Skin regeneration is a complex process involving cutaneous, vascular and immune systems, interconnected by a specific fibroblast pool of growth factors. To determine how this regenerating complex changes with aging and its impact on the functionality of the systems involved in regeneration, an original modeling study was conducted. Results showed that aging has a harmful effect on the metabolism of fibroblasts since aged fibroblasts displayed a reduction in the capacities of migration, differentiation into myofibroblasts and synthesis of a collagen I network, compared to young fibroblasts. Moreover, the study revealed that aging is accompanied by a significant reduction of the fibroblasts capacity to synthesize the set of growth factors. Consequently, the depletion of the fibroblast regenerating complex significantly impedes the functionality of these three major biological systems required for regeneration. Indeed, the treatment by the secretome of aged fibroblasts alters keratinocytes migration, the endothelial cells ability to form a dense and organized vascular network, and the pro-regenerating activities of macrophages. This innovative modeling approach demonstrated the key role of the fibroblast regenerative complex to maintain the interconnectivity and functionality of systems involved in regeneration during aging. Based on these discoveries, we developed a natural active ingredient that revitalizes the interconnectivity of the cutaneous, vascular and immune systems by enhancing the endogenous production of a growth elixir able to reactivate the regeneration process for an anti-aging effect.

Keywords: Skin regeneration; regenerating complex; interconnectivity; immune system; vascular system.

Introduction

Regeneration was for a long time restricted to re-epithelialization depending on keratinocytes and dermal matrix restructuring carried out by fibroblasts [1]. Recent scientific advances shed to light that regeneration is a more complex process requiring the intervention of cutaneous, vascular, and immune systems. Indeed, a population of pro-regenerating macrophages stimulates extracellular matrix remodeling [2], and the vascular network allows the supply to the skin of elements favoring its regeneration like nutrients, cells, and mediators [3]. These three major biological systems are interconnected by the fibroblast, which orchestrates the stages of the regenerating process through the secretion of a specific pool of growth factors. This latter positively regulates the activity of keratinocytes, fibroblasts, endothelial and immune cells, for an optimal and global regenerating effect [4,5]. To this date, evolutions of cutaneous, immune and vascular interconnectivity in regeneration in the course of aging is poorly described. So, the objective of this study was to evaluate modifications of the fibroblast regenerating complex and the consequences on cutaneous, immune and vascular systems in the course of aging.

Materials and Methods.

Studies of fibroblasts metabolism and functionality were conducted on young human fibroblasts from facial plasties and on aged human fibroblasts obtained by successive replications. Changes in the composition of the regenerating complex were studied through the analysis of the expression of EGF, IGF-1 and PDGFA by quantitative PCR and of the synthesis of TGF-β1, KGF, VEGF and FGF2 by ELISA assay. This study was completed by the analysis of three fibroblasts functionalities. Their ability to recolonize a wounded area was followed by time-lapse microscopy, their differentiation into myofibroblasts was evaluated through the expression of alpha smooth muscle actin (α-SMA) and their matrix-remodeling activities were followed by an imunocytofluorescence labeling of collagen I network. The impact of the regenerating complex modifications with aging on key systems of skin regeneration was next evaluated by various biological assays. Modification of the ability of old keratinocytes, obtained from aged donors, treated by secretome of aged fibroblasts to migrate and recolonize a wounded area was followed by time-lapse microscopy. Pro-regenerating activities of the immune system were evaluated by studying

by flow cytometry the marker CD163, specific marker of the pro-regenerating macrophages. Finally, the impact of the secretome of aged fibroblasts on aged endothelial cells pro-regenerating activities was evaluated by the analysis of their abilities to recolonize a wounded area and to establish strong cohesive junctions by following the expression of zonula occludens 1 (ZO-1). The results were compared either with a Student's t test for independent data following a normal law (Shapiro-Wilk test of normal distribution more than 5%) or with a non-parametric Mann-Whitney test for data not normally distributed. Results were compared with a two-tailed test for modeling studies and a one-tailed test for efficacy. In both cases, the significance threshold was set at 5%.

Results.

The analysis of the impact of aging on the composition of the fibroblast regenerating complex revealed a reduced capacity of aged fibroblasts to express or synthesize all growth factors. The results showed a significant decrease of KGF by 40%, EGF by 52%, IGF-1 by 64%, FGF2 by 35%, PDGFA by 41%, TGF-β1 by 23% and VEGF by 35% in the secretome of aged fibroblasts in comparison to young fibroblasts (Fig-1).

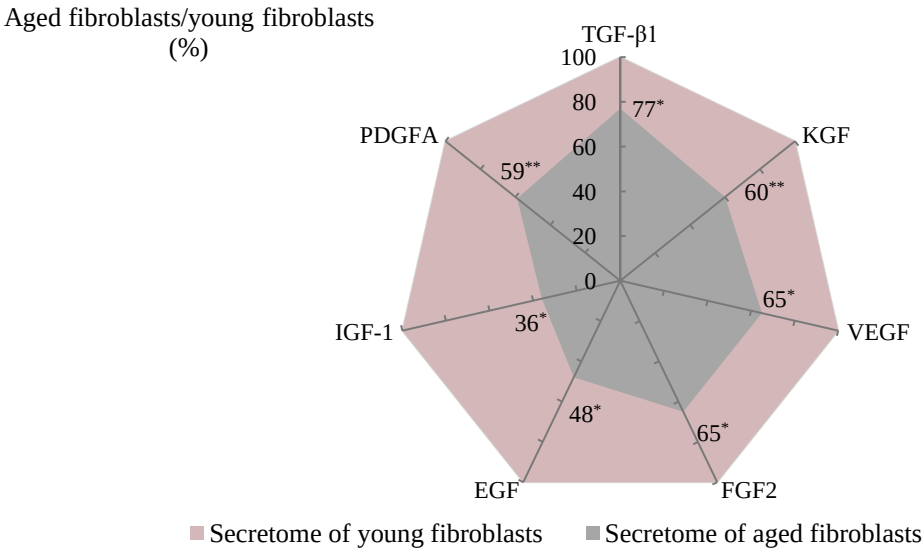
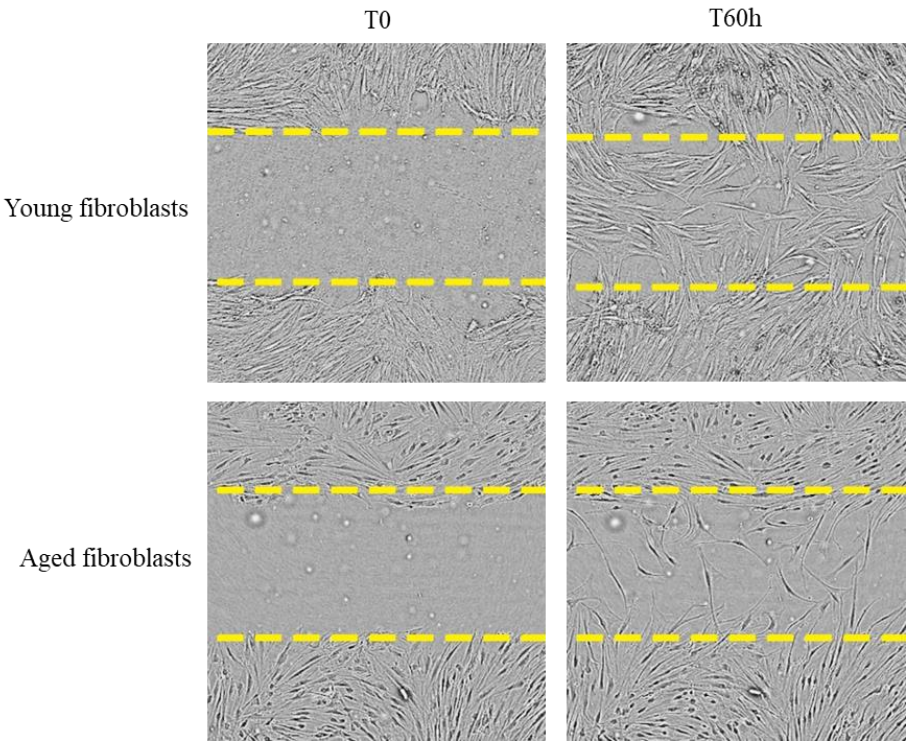


Fig-1. Changes in the levels of growth factors composing the fibroblast regenerating complex with aging (* P<0.05; ** P<0.01).

Moreover, results also showed that aging has a harmful effect on the metabolism of fibroblasts since aged fibroblasts displayed a reduction in the capacities of migration by 27%

(P<0.05) (Fig-2A), differentiation into myofibroblasts by 75% (P<0.001) (Fig-2B) and synthesis of collagen I network by 43% (P<0.001) (Fig-2C), compared to young fibroblasts.

A.



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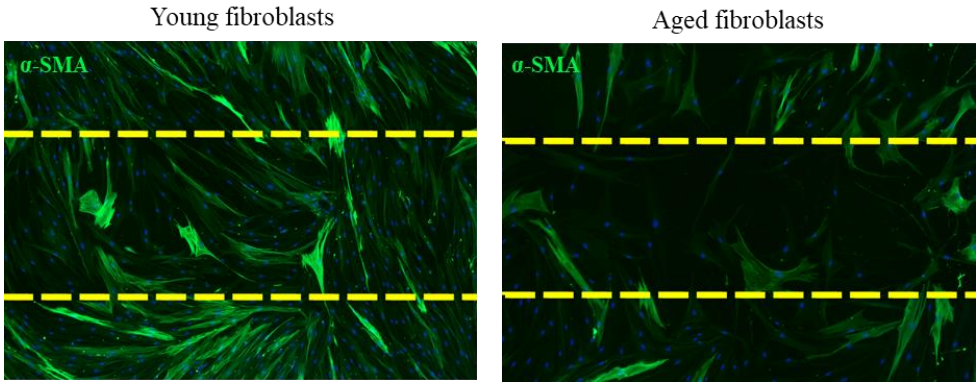

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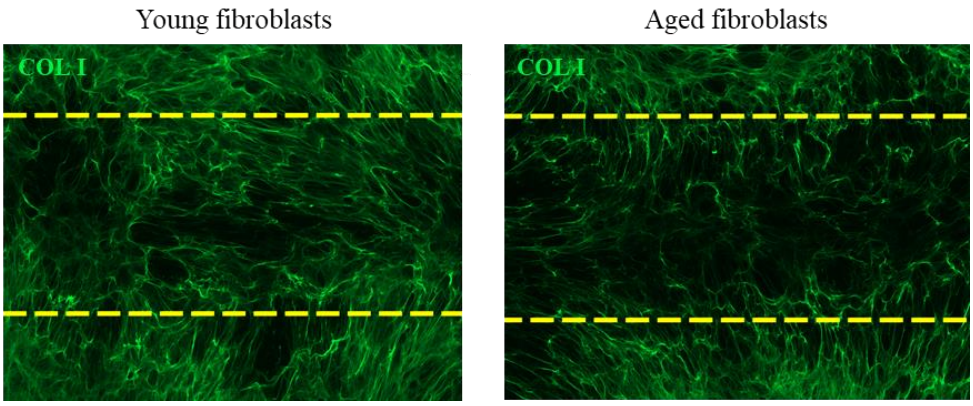


Fig-2. Impairment of fibroblast pro-regenerating activities with aging. A. Reduction in the capacity to recolonize a wounded aera after 60 hours. B. Decrease of the expression of the α-SMA protein. C. Decrease of the synthesis of a collagen I network.

The next part of the study aimed at determining the impact of the depletion of the regenerating complex in growth factors on the functionality of the other cell types of the three systems. We made a first focus on how the dermis influences the epidermis and more particularly the activity of keratinocytes. Migration experiments demonstrated that the depletion in growth factors significantly impedes the ability of old keratinocytes treated with the secretome of aged fibroblasts to recolonize a wounded area by 45% ($P<0.01$), compared to young keratinocytes (Fig-3).

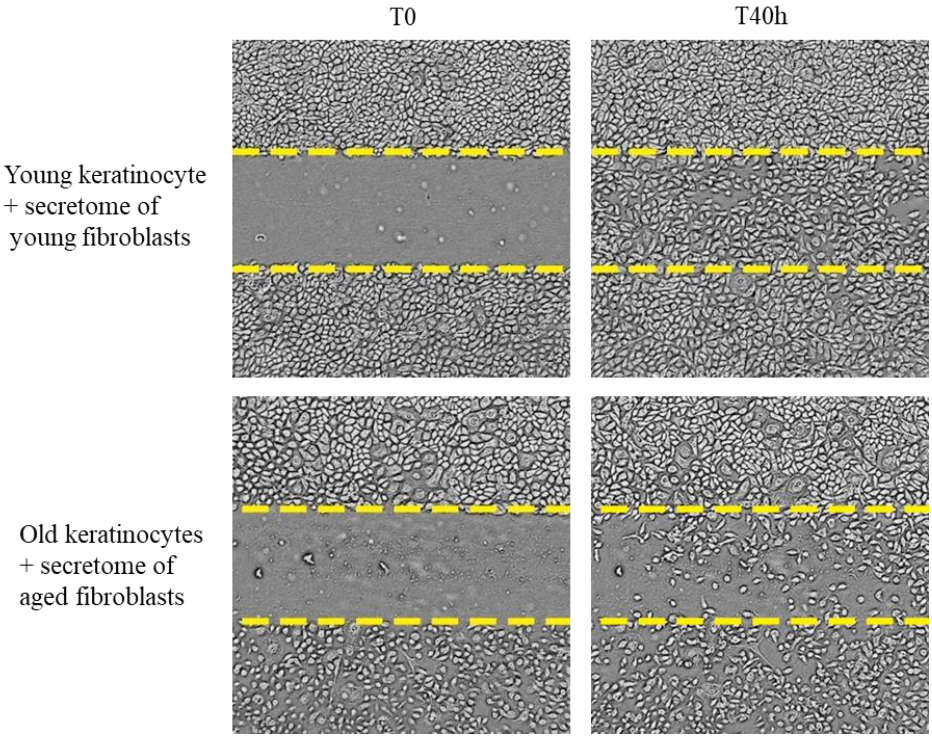


Fig-3. Impact of the secretome of aged fibroblasts on the capacity of keratinocytes to recolonize a wounded area after 40 hours.

We then studied the influence of the dermis on the immune system during aging and more precisely the impact of the depletion in growth factors on the capacity of macrophages to differentiate into pro-regenerative macrophages. Results showed that the exhaustion of the regenerating complex has also a negative impact on the pro-regenerating population of the immune system. Macrophages treated with the aged secretome significantly lose their ability to differentiate into pro-regenerating macrophages by 23% ($P<0.01$) (Fig-4).

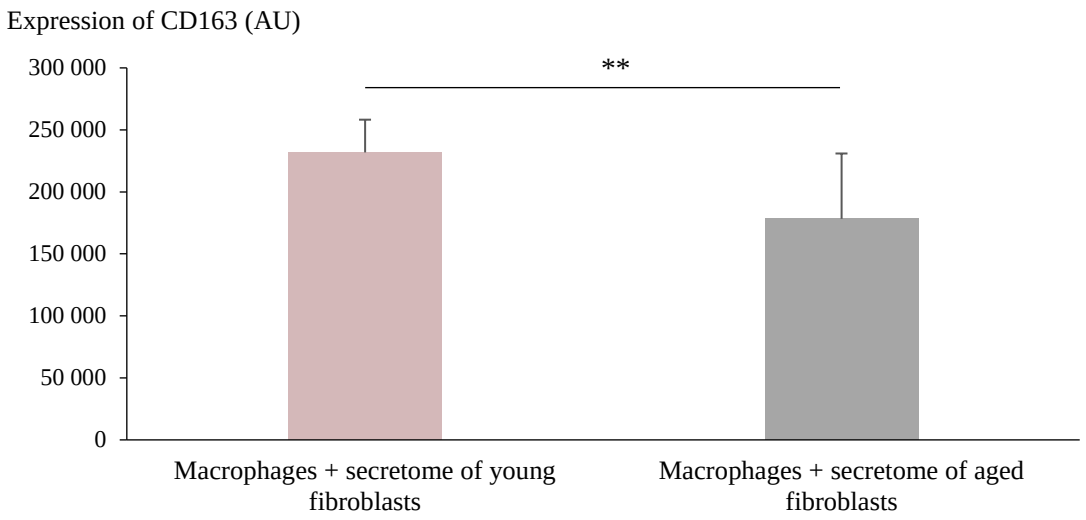
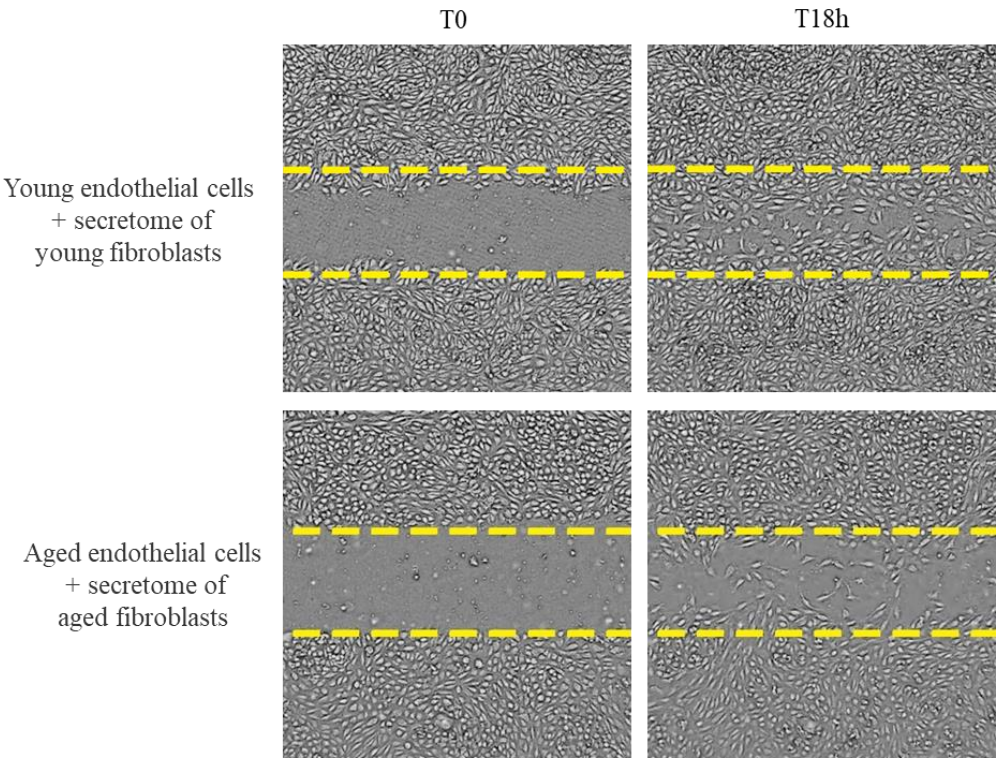


Fig-4. Decrease of the pro-regenerating population of macrophages after the treatment with the secretome of aged fibroblasts (Expression of CD163, ** P<0.01).

Finally, we studied the influence of dermis on the vascular system with aging and more precisely the impact of the depletion of the regenerating complex in growth factors on the vascularization process. One of the prerequisites for the formation of new vessels is the migration of endothelial cells, since they have to group together to assemble. Results showed that in response to treatment with the secretome of aged fibroblasts, the migration capacities of aged endothelial cells were impaired by 47% (P<0.001) (Fig-5A). Moreover, their assembly requires the establishment of strong tight junctions between them, mostly of the ZO-1 type, to guarantee the integrity of the vascular network that they form as they assemble. Results showed that in the aged model the cohesion of endothelial cells is significantly reduced by 52% (P<0.001), demonstrating the negative impact of the aged secretome on the integrity of the vascular system (Fig-5B).

A.



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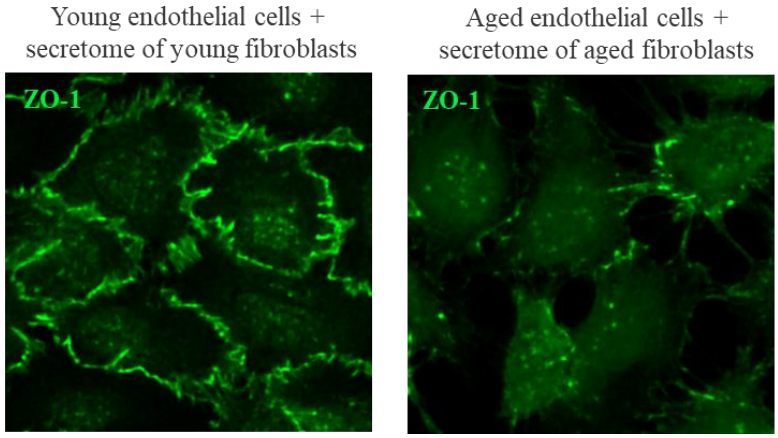

Fig-5. Negative impact of the depletion of the fibroblast regenerating complex on vascularization steps. A. Alteration of the capacity of aged endothelial cells treated with the aged secretome to recolonize a wounded aera after 18h. B. Decrease of the expression of the protein ZO-1 by aged endothelial cells treated with the aged secretome.

Discussion.

This work demonstrated that in comparison to young cells, aged fibroblasts exhibit a significant reduced capacity to express and synthesize the pool of growth factors composing the fibroblast regenerating complex. This depletion in growth factors is accompanied by a drastic alteration of their dermal restructuring-associated activities (migration, differentiation in myofibroblasts and synthesis of collagen I network).

The second part of the work highlighted that the exhaustion of the regenerating complex in growth factors has a profound impact on the interactions of fibroblasts with their environment. Indeed, the interconnectivity of the cutaneous, immune and vascular systems is disrupted and this has deleterious functional consequences on : 1/ the migration abilities of keratinocytes, 2/ the pro-regenerating activity of macrophages, 3/ on the activity of endothelial cells and the cohesion of the vascular system, leading to regeneration defects and consequently to skin aging.

Conclusion.

This work emphasizes the key role of this regenerative complex in maintaining interconnectivity and functionality of cutaneous, immune and vascular systems during regeneration process with aging. Based on these discoveries, SILAB has developed a natural active ingredient composed of oligo-glucans from *Saccharomyces cerevisiae* that revitalizes the interconnectivity of the three biological systems by the endogenous production of a growth elixir able to reactivate the regeneration process for an anti-aging effect.

Conflict of Interest Statement.

NONE.

References.

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