Fibroblast Evaluation: Extracellular Matrix Synthesis

90

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Contents

1 **ECM and Skin Culture Models** 897
2 **ECM in Skin Aging** 898
3 **ECM in Wound Healing and Fibrosis** 899
4 **Conclusion** 900
References 901

Keywords
Skin • Fibroblast • Culture • Extracellular matrix

1 ECM and Skin Culture Models

Much of knowledge on fibroblast physiology is based on monolayer culture studies. After few passages of cells grown from a skin biopsy, fibroblasts exhibit their phenotypic and metabolic characteristics. Thus, cultured papillary and reticular fibroblasts exhibit stable differences in the production of some, but not all, extracellular matrix molecules (Sorrell and Caplan 2004) (Table 1).

To study cell-cell and cell-matrix interactions, three-dimensional organotypic cultures are used. Bell et al. (1979) introduced equivalent dermis models, so-called lattices, in which fibroblasts are embedded into different collagen gel systems. Free-floating lattices retract over time as fibroblasts migrate and reorganize the collagenous matrix. Tense lattices develop internal tension as gel retraction is prevented by attachment of the matrix (Viennet et al. 2005). Such models have revealed the importance of fibroblast adherence to the matrix through mechano-sensing integrin signaling (Dallon and Ehrlich 2008). When integrins bind collagen, they transduce a signal which regulates cellular biological functions, such as proliferation, migration, and matrix metabolism. Intracellular signaling cascades such as

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Table 1 Expression of extracellular matrix molecules by monolayer cultures of dermal fibroblasts (from Sorrell and Caplan 2004)

Matrix component	Papillary fibroblasts	Reticular fibroblasts
Collagens I and III	Produced – ratio same as for reticular cells	Produced – ratio same as for papillary cells
Collagens V and VI	Produced	Produced
Collagen XII	Produced	Produced
Collagen XIV	Not produced in monolayer culture	Not produced in monolayer culture
Collagen XVI	Produced at high levels	Produced at low levels
Tenascin-C	Produced	Produced
Tenascin-X	Not studied	Not studied
Versican	Produced at low levels	Produced at high levels
Decorin	Produced at high levels	Produced at low levels

transforming growth factor-β (TGF-β), extracellular signal-regulated kinases, p38 mitogen-activated protein kinase, and Wnt/β-catenin pathway have been implicated in the regulation of ECM production in fibroblasts.

Fibroblasts synthesize matrix metalloproteinases (MMPs), which are responsible for the turnover of collagen and elastic fibers. MMPs are zinc-dependent enzymes involved in the degradation of ECM and in the release and activation of growth factors and cytokines. Their activity is controlled by tissue inhibitors of metalloproteinases (TIMPs), and their production is regulated by a number of factors, including cell/ECM interaction, mechanical force, and the expression of cytokines/growth factors (TGF-β, IL-1, TNFα). According to the MMPs expression profile, it is possible to characterize different subtypes of fibroblasts: oral fibroblasts exhibit increased levels of the active form of MMP-2 as compared to dermal fibroblasts (Stephens et al. 2001). Expression or activation of MMPs differs if fibroblasts are cultured in monolayer or in three-dimensional collagen lattice. MMP activity is significantly induced when keratinocytes and fibroblasts were cocultured. Paracrine interaction between keratinocytes and fibroblasts appears to play an important role in the regulation of collagen metabolism (Tandara and Mustoe 2011).

Gene expression and synthesis of ECM molecules in biological models of the skin can be measured by PCR, zymography, and ELISA.

Due to diverse synthetic functions, fibroblast activities need control. Improper control results in synthesis of excessive or inadequate products that are reflected in disease processes such as scleroderma, systemic sclerosis, and keloids, but also in wrinkles and premature aging of the skin.

2 ECM in Skin Aging

With aging, fibroblasts lose their proliferative potential, and they show diminished ECM biosynthesis capacity, resulting in dermal atrophy and wrinkle formation. Compared to adjacent skin fibroblasts, wrinkle-cultured fibroblasts show decreased production of type I collagen and increased synthesis of MMP-1 (Fig. 1). In monolayer culture, fibroblasts from old donors, or even in vitro aged fibroblasts, have been shown to express age-specific features. Aged fibroblasts show a decrease in proliferation and type I collagen production and an increase in MMP-1 synthesis compared to young ones (Humbert et al. 2012).

One of the causes of aging is the appearance of the advanced glycosylation end products (AGEs) during life. The glycation reaction results from a nonenzymatic reaction between a sugar and a free amine group of Lys, Arg amino acids in proteins. Therefore, it seems that AGEs modify the expression and the synthesis of ECM molecules. They provoke the activation of fibroblasts; the increase of type III and IV collagens; MMP-1, MMP-2, and MMP-9 production; and the modification of α6 and β1 integrin patterns (Pageon et al. 2007; Pageon 2010) (Fig. 2).

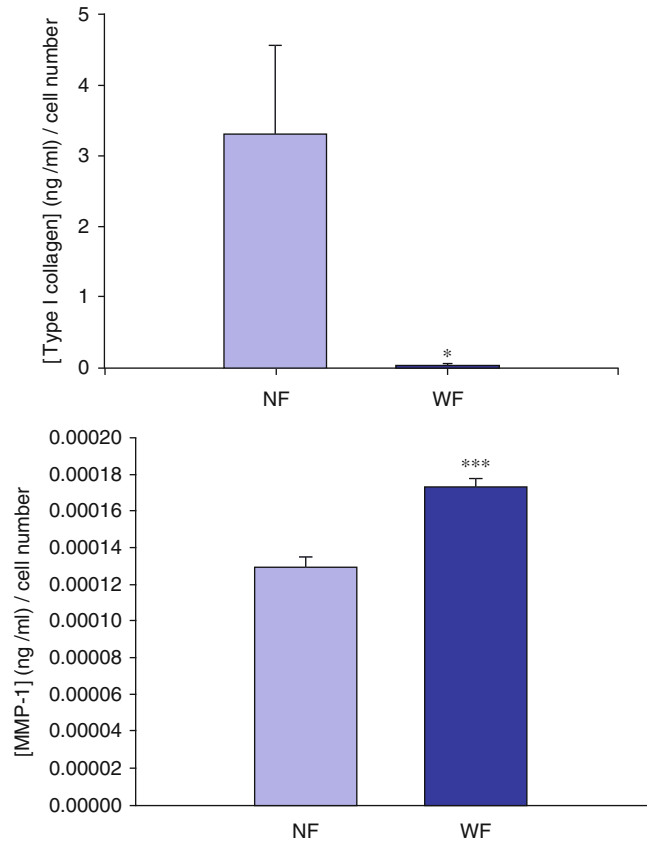

Fig. 1 Expression of type I collagen and MMP-1 molecules by monolayer cultures of adjacent skin (*NF*) and wrinkle fibroblasts (*WF*) (Unpublished data)

3 ECM in Wound Healing and Fibrosis

Wound healing is a complex process that involves inflammation, granulation tissue formation, and tissue remodeling, and this is all regulated by a number of cytokines and growth factors. Fibroblasts play a crucial role in wound healing. During this process, fibroblasts proliferate and produce many of the ECM molecules in a TGF-β-dependent manner, and this contributes to the recovery of connective tissue integrity.

The expression by dermal fibroblasts of several genes and the synthesis of proteins involved in skin wound healing were found to be tension inducible (Agha et al. 2011). This is the case of type I and III collagens and procollagen C-proteinase (enzyme required for the formation of insoluble collagen fibers) whose expression is increased by mechanical tension (Lambert et al. 2001; Kessler et al. 2001). Other ECM components are also induced by mechanical tension such as Tenascin-C, a modulator of cell adhesion, migration, and growth (Chiquet-Ehrismann and Chiquet 2003). As regards the remodeling of ECM, MMP-1, MMP-3, MMP-9, and MMP-13 are upregulated when tensile forces are dissipated (Lambert et al. 2001).

Disruption of either production or degradation of ECM and imbalances between MMPs and TIMPs production can lead to abnormal scarring. Both keloids and hypertrophic scars are cutaneous abnormalities that are characterized by excessive accumulation of connective tissue, especially collagen. When compared with normal fibroblasts, keloid fibroblasts exhibit an altered expression of several molecules implied in all wound healing phases. Cultured keloid fibroblasts showed increased production of type I collagen,

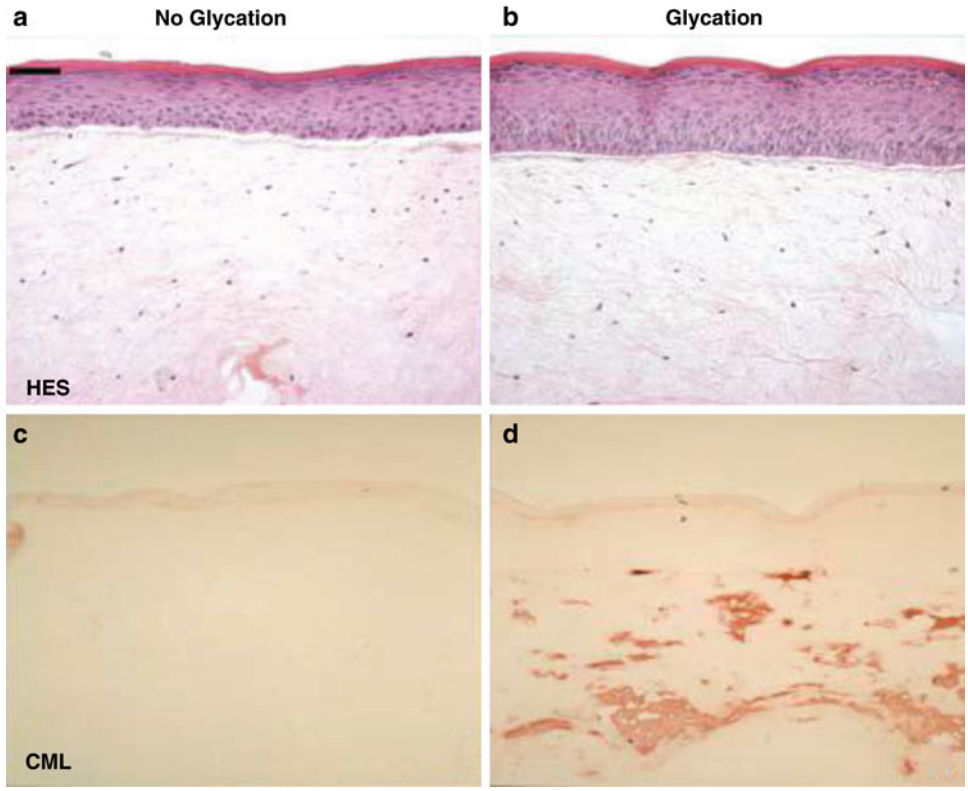

Fig. 2 HES histological staining (**a**, **b**) and AGE labeling (**c**, **d**) of skin reconstructed in vitro using untreated collagen (**a**, **c**) or preglycated collagen (**b**, **d**). The histological pictures look similar except that the dermal organization seems to be modified by glycation (bar = 100 μm) (From Pageon et al. 2007)

fibronectin, MMP-1, MMP-2, MMP-3, MMP-13, MMP-19, and TIMP-1. MMP-8 is decreased (Shih et al. 2010). In addition, increased production of MMPs had a role in the high migratory activity of cultured keloid fibroblasts (Fujiwara et al. 2005).

Systemic scleroderma is a generalized disorder characterized by excessive deposition of ECM. The overproduction of type I, III, and VI collagens by systemic and localized scleroderma fibroblasts has been demonstrated (Krieg et al. 1981). Increased matrix deposition has been shown to be due to enhanced gene transcription and stability of mRNA. In comparison with normal fibroblasts, systemic scleroderma fibroblasts respond differently to their extracellular environment, indicating an altered feedback (Herzhoff et al. 1999).

4 Conclusion

The extracellular matrix is one of the most important regulators of cellular and tissue function in the body. Tightly controlled ECM homeostasis is essential for development, wound healing, and normal organ homeostasis, and dysregulation can result in pathological conditions. Fibroblasts are the major cells in the dermis, and these cells provide tensile strength and elasticity through the production and secretion of various components of ECM, including collagens, elastin, glycoproteins, and proteoglycans. Thus enhancing the activity of fibroblasts, in the context of ECM production, may have beneficial effects on maintenance of skin texture.

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