# Fibroblast heterogeneity: more than skin deep

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## Summary

**Dermal fibroblasts are a dynamic and diverse population of cells whose functions in skin in many respects remain unknown. Normal adult human skin contains at least three distinct subpopulations of fibroblasts, which occupy unique niches in the dermis. Fibroblasts from each of these niches exhibit distinctive differences when cultured separately. Specific differences in fibroblast physiology are evident in papillary dermal fibroblasts, which reside in the superficial dermis, and reticular fibroblasts, which reside in the deep dermis. Both of these subpopulations of fibroblasts differ from the fibroblasts that are associated with hair follicles. Fibroblasts engage in fibroblast-epidermal interactions during hair development and in interfollicular regions of skin. They also play an important role in cutaneous wound repair and an ever-increasing role in bioengineering of skin. Bioengineered skin currently performs important roles in providing (1) a basic understanding of skin biology, (2) a vehicle for testing topically applied products and (3) a resource for skin replacement.**

Key words: Skin, Fibroblasts, Skin equivalents

## Introduction

Dermal fibroblasts are an essential component of skin; they not only produce and organize the extracellular matrix of the dermis but they also communicate with each other and other cell types, playing a crucial role in regulating skin physiology. Other resident cells include epidermal, vascular and neural cells (Ansel et al., 1996; Detmar, 1996; Werner and Smola, 2001). In addition, skin contains various cells of hematopoietic origin. These include a constitutive population of dendritic cells and a more ephemeral population of leukocytes that includes monocytes/macrophages, neutrophils and lymphocytes (Nestle and Nickoloff, 1995; Gonzalez-Ramos et al., 1996; Lugovic et al., 2001). Dermal fibroblasts represent a heterogeneous population of cells defined according to their location within the dermis (Fig. 1). Two subpopulations of fibroblasts reside in distinct dermal layers: the papillary and reticular dermis (Cormack, 1987). Fibroblasts cultured from each of these layers have different characteristics (Harper and Grove, 1979; Azzerone and Macieira-Coelho, 1982; Schafer et al., 1985; Sorrell et al., 1996; Sorrell et al., 2004). A third group is associated with hair follicles. These lie in the dermal papilla region of the follicle and along its shaft (Reynolds and Jahoda, 1991; Jahoda and Reynolds, 1996). Other subpopulations of dermal fibroblasts might also exist; however, the focus of this Commentary is the fibroblast subpopulations that exhibit stable and well-characterized differences in culture.

## Papillary and reticular dermal fibroblasts

The papillary dermis is approximately 300-400 μm deep. This depth is variable and depends upon such factors as age and anatomical location. Typically, the superficial portion of the papillary dermis is arranged into ridge-like structures, the dermal papillae, which contain microvascular and neural components that sustain the epidermis (Cormack, 1987). Dermal papillae greatly extend the surface area for epithelial-mesenchymal interactions and delivery of soluble molecules to the epidermis. A vascular plexus, the rete subpapillare, demarcates the lower limit of the papillary dermis (Figs 1, 2). The reticular layer of the dermis extends from this superficial vascular plexus to a deeper vascular plexus, the rete cutaneum, which serves as the boundary between the dermis and hypodermis. Hair follicles and their associated dermal cells extend into and often through the reticular dermis to terminate in the hypodermis, a tissue rich in adipocytes.

Mechanical separation of skin (dermatoming) into defined papillary and reticular layers allows establishment of explant cultures of cells from each layer. Papillary fibroblasts divide at faster rates than do site-matched reticular fibroblasts (Harper and Grove, 1979; Azzerone and Macieira-Coelho, 1982; Schafer et al., 1985; Sorrell et al., 1996; Sorrell et al., 2004). Reticular dermal fibroblasts seeded into type I collagen lattices contract them faster than do papillary dermal fibroblasts (Schafer et al., 1985; Sorrell et al., 1996). When grown to confluence in monolayer culture, the papillary cells attain a higher cell density partly because they are not fully contact inhibited (Schafer et al., 1985; Sorrell et al., 2004).

## Extracellular matrix differences

The papillary dermis and reticular dermis differ in both the composition and organization of their respective extracellular matrices (Table 1). The papillary dermis is characterized by thin, poorly organized collagen fiber bundles, consisting primarily of type I and type III collagens, which contrast with the thick, well-organized fiber bundles in the reticular dermis (Cormack, 1987). Collagen fiber bundles in the papillary dermis contain more type III collagen than do those in the reticular dermis (Meigel et al., 1977). Other matrix molecules are also differentially apportioned between the papillary and reticular dermis. Immunohistochemical studies of normal adult

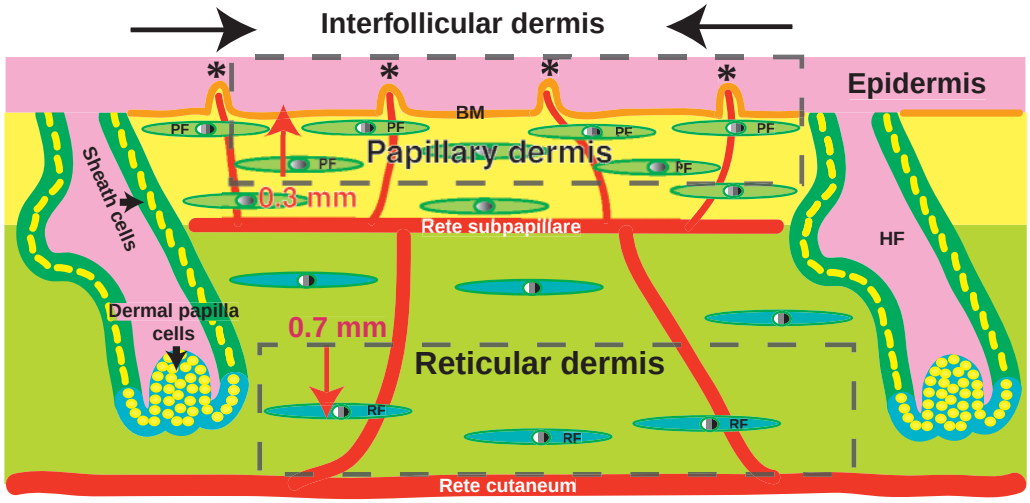


**Fig. 1.** Adult human skin is a layered organ consisting of an epidermis that is attached to a dermis by an elaborate connective tissue structure, the basement membrane (BM). The basal surface of the epidermis is indented by dermal and vascular components called dermal papillae (*). The dermis is divided into two functional layers, the papillary dermis and reticular dermis. These two layers are separated by a vascular plexus, the rete subpapillare. This plexus is fed by another vascular plexus, the rete cutaneum, located at the base of the reticular dermis. Skin also contains hair follicles (HF) and glands (not shown). Two distinct populations of dermal fibroblasts have been cultured from the interfollicular dermis, the region between hair follicles. Papillary fibroblasts (PF) are cultured from skin dermatomed at a depth of 0.3 mm and reticular fibroblasts (RF) are cultured from skin located at a depth below 0.7 mm. Hair follicle fibroblasts are obtained by carefully plucking or dissecting hairs from the skin and then placing these hairs or segments of these hair follicles onto surfaces of plastic culture dishes. Hair follicles contain two subsets of cells: the follicular sheath cells and dermal papilla cells.

skin highlight structural and compositional differences in proteoglycan deposition (Fig. 3). The proteoglycan decorin is intensely expressed in the papillary dermis, but is otherwise dispersed between collagen fiber bundles in the reticular dermis. By contrast, versican associates with microfibrils in the papillary dermis, but is more extensively expressed in elastic fibers of the reticular dermis (Zimmermann et al., 1994; Sorrell et al., 1999a). The non-fibrillar collagen types XII and XVI, along with tenascin-C, are characteristically found in the papillary dermis; whereas, collagen type IV and tenascin-X are primarily restricted to the reticular dermis (Lightner et al., 1993; Wälchli et al., 1994; Lethias et al., 1996; Berthod et al., 1997; Akagi et al., 1999; Grässel et al., 1999).

Experimental studies have explored the issue of whether cultured papillary and reticular fibroblasts produce different amounts and types of extracellular matrix molecule that might account for the observed differences in skin (Table 2). In monolayer cultures, Schönherr et al. found that papillary dermal fibroblasts secrete significantly more decorin than did corresponding reticular cells, and papillary fibroblasts contain more decorin mRNA (Schönherr et al., 1993). By contrast, the two cellular populations produce identical amounts of

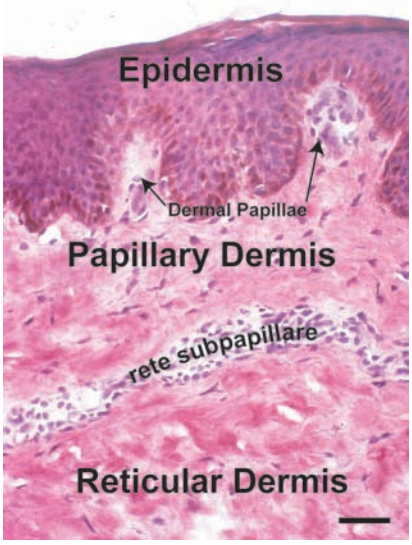


**Fig. 2.** The papillary and reticular dermis is separated by a vascular plexus, the rete subpapillare. The papillary dermis contains a higher density of cells than does the reticular dermis. Dermal papillae extend the surface area of the epithelial-mesenchymal boundary. Bar, 45 μm.

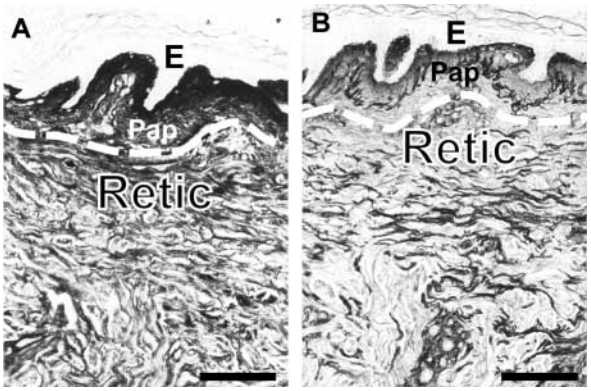


**Fig. 3.** Immunohistochemical studies indicate that the papillary dermis (Pap) contains high levels of the proteoglycan decorin (A). The reticular dermis (Retic) contains elastic fibers oriented parallel to the epidermis that contain the proteoglycan versican. Microfibrils containing versican are also present in the papillary dermis as is diffuse versican at the DEJ (panel B). The epidermis (E) does not contain detectable levels of these two proteoglycans. The dashed line indicates the approximate demarcation between the papillary and reticular layers (adapted from Sorrell et al., 1999a, with kind permission from Kluwer Academic Publishers). Bar, 87 μm.

**Table 1. Distribution of selected extracellular matrix molecules in dermal compartments**

| Matrix component | Papillary dermis | Reticular dermis | Hair follicle |
|---|---|---|---|
| Collagens I and III | High ratio of type III to I | Low ratio of type III to I | Present |
| Collagen IV | Present in basement membrane | Absent | Present in dermal papillae |
| Collagen VI | Present at dermal-epidermal junction (DEJ) | Weakly present | Present in dermal sheaths |
| Collagen XII | Present | Low to absent | High expression around follicular sheath |
| Collagen XIV | Low to absent | Present | Low expression |
| Collagen XVI | Present in DEJ-region | Absent | Unknown |
| Tenascin-C | Present in DEJ-region | Absent | Present in sheaths and dermal papillae |
| Tenascin-X | Weak in DEJ-region | Present | Not associated |
| Versican | Diffuse in DEJ-region, present in matrix fibrils | Present in association with elastic fibers | Present in dermal papillae |
| Decorin | Present | Present | Unknown |

biglycan. Another study found that site-matched papillary and reticular fibroblasts differ in the relative levels of the proteoglycans decorin and versican that they produce (Sorrell et al., 1999b).

Akagi et al. found that fibroblasts derived from the upper, middle and lower thirds of the dermis produced significantly different amounts of mRNA for the $\alpha_1$(XVI) of type XVI collagen (Akagi et al., 1999). By contrast, Tajima and Pinnell quantified the amounts of type I and type III collagens produced by monolayer cultures to see whether synthetic differences might account for the observed in vivo differences (Tajima and Pinnell, 1981). They found no differences in the production of type I and type III collagens by these two populations of cultured cells, although they noted an elevated amount of type I procollagen in the medium of papillary fibroblast cultures. Thus, cultured papillary and reticular fibroblasts exhibit stable differences in the production of some, but not all, extracellular matrix molecules.

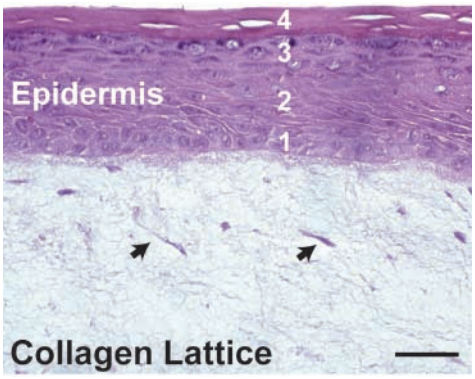


**Fig. 4.** A skin equivalent consists minimally of a dermal equivalent and differentiated epidermis cultured first submerged then at the air-liquid interface in a three-dimensional context. Fibroblasts (arrows) encased in a type I collagen lattice provide dermal support for the epidermis. The epidermis is stratified and contains differentiated layers typically found in normal skin, including the (1) basal, (2) spinous, (3) granular and (4) cornified layers. Bar, 44 μm.

## Fibroblasts and basement membrane formation

The epidermis of the skin is firmly attached to the underlying dermis by a complex multi-molecular structure, the basement membrane (Burgeson and Christiano, 1997; Aumailley and Rousselle, 1999). The organization of basement membrane to form a morphologically identifiable structure results from a cooperative effort of both keratinocytes and fibroblasts (Fleischmajer et al., 1993; Marinkovich et al., 1993; Smola et al., 1998; Moulin et al., 2000). Marinkovich et al. studied the cellular origin of various basement membrane molecules by probing skin equivalents (Fig. 4) that contain bovine keratinocytes and human dermal fibroblasts with species-specific antibodies (Marinkovich et al., 1993). Type IV and VII collagen and laminin-1 produced by fibroblasts appeared in a linear array at the dermal-epidermal junction (DEJ). Keratinocytes also produced and organized type IV and VII collagen, laminin-5, other laminins and perlecan. Other studies have shown that fibroblasts are the principal source of entactin/nidogen (Contard et al., 1993; Fleischmajer et al., 1995). Marinkovich et al. then postulated that a differentiated population of fibroblasts exists at the DEJ of skin that both produces basement membrane components and helps keratinocytes organize them (Marinkovich et al., 1993).

Coculture of fibroblasts and keratinocytes modifies the activities of both cell types. Keratinocytes induce the expression of transforming growth factor (TGF)-β2 by dermal fibroblasts (Smola et al., 1994). Fibroblasts regulate the production of laminins and type VII collagen by keratinocytes, possibly through TGF-β signaling (König and Bruckner-Tuderman, 1991; König and Bruckner-Tuderman, 1994;

**Table 2. Expression of extracellular matrix molecules by monolayer cultures of dermal fibroblasts**

| 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 |

Monical and Kefalides, 1994). The kinetics of basement membrane formation has also been studied in organotypic coculture models in which fibroblasts were either present or omitted (Smola et al., 1998). Specific basement membrane components gradually appeared at the DEJ; however, the kinetics varied, depending on whether fibroblasts were present. The production of type IV collagen, laminin-1 and type VII collagen by keratinocytes cultured alone was significantly delayed or absent, suggesting that fibroblasts influenced the production of these matrix molecules. On the dermal side, the steady-state mRNA levels of type IV collagen α1 message in fibroblasts were significantly enhanced when keratinocytes were present. Together, these studies indicate that elements of basement membrane production are co-regulated by fibroblasts and keratinocytes.

Not all dermal fibroblasts interact equally well with keratinocytes in the formation of a basement membrane. Moulin et al. showed that myofibroblasts obtained from wound sites do not support keratinocyte differentiation and basement membrane formation to the same extent as do normal dermal fibroblasts (Moulin et al., 2000). Consequently, the ability was compared of site-matched papillary and reticular dermal fibroblasts to support basement membrane formation (Sorrell et al., 2004). Papillary dermal fibroblasts appeared to induce basement membrane formation faster when reticular fibroblasts were present. Therefore, fibroblasts adjacent to the epidermis might either produce more extracellular matrix components of the basement membrane and/or produce soluble factors that influence keratinocytes to re-establish a basement membrane.

## Intercellular communication and interfollicular dermal fibroblasts

Fibroblasts engage in paracrine and autocrine interactions in skin (Gilchrest et al., 1983; Boxman et al., 1993; Smola et al., 1993; Kupper and Groves, 1995; Moulin, 1995; Schröder, 1995; Slavin, 1996; Smith et al., 1997; Kondo, 2000; Werner and Smola, 2001). Rheinwald and Green developed a culture system in which irradiated mesenchymal cells support the growth of adult human keratinocytes (Rheinwald and Green, 1975). This led to the identification of mesenchyme-derived factors that regulate keratinocyte proliferation, including keratinocyte growth factor (KGF)-1. This is a member of the fibroblast growth factor (FGF) family that is exclusively produced by mesenchymal cells (Rubin et al., 1995; Werner, 1998). However, only epithelial cells express the KGF receptor and, hence, respond to KGF-1. Fibroblasts also produce other factors that regulate the proliferation of cultured keratinocytes and play roles in wound repair. These include granulocyte-macrophage colony-stimulating factor (GM-CSF), FGF-10 (also known as KGF-2), parathyroid-hormone-related protein, hepatocyte growth factor/scatter factor (HGF/SF), epidermal growth factor (EGF) and interleukin 6 (IL-6) (Waelti et al., 1992; Boxman et al., 1993; Rubin et al., 1993; Smola et al., 1993; Sato et al., 1995; Igarashi et al., 1996; Blomme et al., 1999; Breuhahn et al., 2000; Mann et al., 2001; Marchese et al., 2001; Werner and Smola, 2001).

Fibroblasts release growth factors/cytokines that play a significant role in wound repair by modulating the activity of keratinocytes. Smola et al. found that coculture of fibroblasts and keratinocytes results in increased levels of KGF-1, IL-6 and GM-CSF mRNAs (Smola et al., 1993). The level of KGF-1 mRNA and the amount of protein released into culture medium by cultured dermal fibroblasts were upregulated by treatment of these cells with IL-1 (Brauchle et al., 1994; Chedid et al., 1994; Maas-Szabowski and Fusenig, 1996). KGF-1 in turn enhanced the release of IL-1α by keratinocytes. Thus, a paracrine loop is established in situations where dermal fibroblasts and keratinocytes coexist (Maas-Szabowski et al., 1999).

Soluble factors released by fibroblasts do not possess inductive characteristics with respect to interfollicular keratinocytes. Nonetheless, these factors can modulate specific aspects of epidermal formation. Overexpression of KGF-1 results in a hyperproliferative epidermis. This might result from enhanced proliferation of basal keratinocytes and suppression of terminal differentiation (Guo et al., 1993; Hines and Allen-Hoffmann, 1996; Szabowski et al., 2000; Andreadis et al., 2001). Excessive KGF-1 might also induce flattening of the basal surface of the epidermis (Andreadis et al., 2001). By contrast, overexpression of GM-CSF results in increased apoptosis of cultured keratinocytes, and overexpression of KGF-2 could accelerate keratinocyte differentiation (Breuhahn et al., 2000; Suzuki et al., 2000; Marchese et al., 2001). These observations have led to the proposal that the epidermal response to fibroblast-derived signaling molecules depends upon the ratio of these factors. Fusenig and coworkers have proposed that the ratio of KGF-1 to GM-CSF presented to epidermal cells determines the status of this tissue (Maas-Szabowski et al., 2001). Site-matched papillary and reticular dermal fibroblasts differ significantly in the release of KGF-1 and GM-CSF into culture medium. Typically, the ratio of GM-CSF to KGF-1 is higher in papillary fibroblasts than in corresponding reticular cells (Sorrell et al., 2004). Thus, these two populations of cells exert subtle differences on epidermal proliferation and differentiation.

Communication between fibroblasts and keratinocytes appears to involve AP-1 target genes in dermal fibroblasts. Szabowski et al. examined fibroblasts from Jun-knockout and JunB-knockout mouse embryos and found that the $Jun^{-/-}$ cells produce very low levels of KGF-1 and GM-CSF, whereas $JunB^{-/-}$ cells produce elevated levels of these factors (Szabowski et al., 2000). Incorporation of these fibroblasts into bi-layered skin equivalents with normal adult human keratinocytes for the epidermal layer led to strikingly different results. Epidermal layers on skin equivalents containing $Jun^{-/-}$ fibroblasts were atrophic, basal cell proliferation was reduced, and terminal differentiation was delayed. $JunB^{-/-}$ fibroblasts caused epidermal hyperplasia. IL-1 and other inflammatory factors, such as tumor necrosis factor (TNF)-α, activate AP-1-mediated transcription and enhance the activity of NF-κB (Angel and Szabowski, 2002). Differences in the phenotypes of fibroblasts in skin might be related to how these cells respond to external signals and modulate the diverse group of genes regulated by AP-1 transcription factors.

## Dermal fibroblastic cells are associated with hair follicles

Hair follicles are skin appendages formed predominantly by cells of epidermal origin. Mesenchymal cells of the dermis play a vital role in their formation in fetal skin and an equally

significant role in regulating their cyclic growth, rest and regression phases in adults (Kulessa et al., 2000; Botchkarev, 2003). In fetal skin, mutual inductive events between localized dermal and epidermal cells proceed in a stringent spatio-temporal manner. First, an as-yet-undefined signal emanating from the dermis induces the formation of thickened epidermal placodes (Holbrook and Minami, 1991; Hardy, 1992; Millar, 2002; Botchkarev et al., 1999; Botchkarev et al., 2002). Differentiated epidermal cells provide a second signal that induces localized mesenchymal cells to condense and form a defined pellet of cells immediately beneath the epidermal placodes (Holbrook and Minami, 1991; Hardy, 1992). These cells stimulate the proliferation of epidermal cells in the placode, which drives the production of hair follicles deep into the dermal matrix (Hardy, 1992; Millar, 2002; Botchkarev, 2003). Simultaneously, condensed mesenchymal cells produce proteases that clear a path for this ingrowth (Karelina et al., 1993; Karelina et al., 1994; Karelina et al., 2000). Once elongation is complete, keratinocytes in the matrix region at the base of the follicle envelop the dermal papilla cells and leave a narrow opening through which the vasculature and nerves penetrate (Hardy, 1992; Millar, 2002; Botchkarev, 2003). Condensed mesenchymal cells also give rise to a second population of dermal cells during the period in which follicles actively invade the dermal matrix. These dermal cells form a thin connective tissue sheath along the shaft of the follicle (Jahoda and Reynolds, 2000).

## The fibroblast in cutaneous wound repair

Fibroblasts play a crucial role in cutaneous wound repair (Martin, 1997). These cells are attracted to wound sites by the localized release of growth factors/cytokines such as platelet-derived growth factor (Pierce et al., 1991). The first wave of fibroblasts enters the wound site along with sprouting vasculature. These cells differentiate into a specialized, but ephemeral, cell type called the myofibroblast (Sappino et al., 1990; Grinnell, 1994).

Myofibroblasts, in response to monocyte/macrophage-derived factors, produce a provisional wound matrix that is enriched in fetal-like fibronectin and hyaluronan (Clark, 1990; Gailit and Clark, 1994; Juhlin, 1997; Singer and Clark, 1999). These cells also provide the motive force to contract the wound (Sappino et al., 1990). Myofibroblasts disappear from the wound site, apparently by apoptosis, and are replaced by a second wave of fibroblasts that initiate the formation of a collagenous matrix (Grinnell et al., 1999). However, their ability to organize it is impaired, which results in the formation of scar tissue (Gailit and Clark, 1994; Shah et al., 1994; Shah et al., 1995; Singer and Clark, 1999). Fetal skin is repaired without scar formation (Adzick and Lorenz, 1994; Armstrong and Ferguson, 1995; Liechty et al., 2000). This is mainly owing to differences in fetal and adult fibroblast phenotypes (Schor et al., 1985; Olsen and Uitto, 1989; Cullen et al., 1997; Gosiewska et al., 2001). The low level of growth factors/cytokine production by fetal cells, especially TGF-β1, appears to be a major factor in the absence of scar formation (Shah et al., 1994; Shah et al., 1995; Eckes et al., 2000). The aberrant fibroblast phenotype also appears to contribute to fibrotic disorders, such as keloid formation and scleroderma (Garner et al., 1993; Ghahary et al., 1994; Ghahary et al., 1996; Sollberg et al., 1994; Kirk et al., 1995; Nakaoka et al., 1995; Herrick et al., 1996; Hasan et al., 1997; Agren et al., 1999). Signals such as TGF-β and connective tissue growth factor play a significant role in the latter process (Grotendorst, 1997).

## The dermal fibroblast in bioengineering

Much of our current knowledge regarding fibroblast physiology is derived from studies of these cells grown on a plastic substrate as monolayer cultures. Fibroblasts cultured in this manner retain many of their phenotypic characteristics (see above). Nonetheless, monolayer-cultured fibroblasts exhibit significant metabolic differences from in vivo fibroblasts. For example, fibroblasts in monolayer culture actively proliferate and produce many different types of extracellular matrix molecule. Both of these characteristics are either suppressed or greatly diminished in three-dimensional organotypic cultures much in the same manner as in vivo (Mauch et al., 1988; Kono et al., 1990; Geesin et al., 1993; Grinnell, 1994; Mio et al., 1996; Ivarsson et al., 1998; Rosenfeldt and Grinnell, 2000).

## Dermal and skin equivalents as biological models

The application of three-dimensional organotypic cultures to tissue-specific modeling studies has undergone significant development (Schmeichel and Bissell, 2003). Dermal and skin equivalents were among the first examples of such organotypic cultures (Bell et al., 1979; Bell et al., 1981; Bell et al., 1983; Asselineau et al., 1986). These culture systems provide a resource for basic studies in skin biology, testing for topically applied products, and as a replacement for human skin. For example, environmental aging of skin due to chronic exposure to UV irradiation poses cosmetic challenges and increased risk of skin cancers (Gilchrest, 1996). Bernerd and Asselineau studied the effects of UV irradiation in a skin equivalent model (Bernerd and Asselineau, 1997). They found that ‘sunburn’ cells were generated in the epidermis by an acute UVB exposure in much the same manner as occurs in skin. Furthermore, downregulation of keratinocyte differentiation markers occurred at early time points following UVB exposure. These situations were repaired in skin equivalents that were maintained in culture for extended periods of time. In another study, they found that UVA exposure induces responses specific to the dermal compartment of skin equivalents (Bernerd and Asselineau, 1998). Fibroblasts in the upper regions of the ‘dermal’ component of the skin equivalents underwent apoptosis and disappeared from the constructs. Over time, fibroblasts at the bottom of the skin equivalent were induced to proliferate and migrated into the upper region of the construct. This was accompanied by an increase in metalloproteinase (MMP)-1 synthesis by resident fibroblasts, which presumably enabled the cells to migrate within the collagen gel.

Michel et al. have investigated skin equivalents as potential tools for percutaneous absorption (Michel et al., 1993). They prepared human skin equivalents such that a constant surface area was present and found that absorption of chemical agents depends on the thickness of the epidermis and its stratum corneum. This process was not entirely equivalent to that observed in mice, but was sufficient to suggest that it might be used as an effective model for pharmacological and cosmetic

testing. Development of skin equivalents that contain other types of cell, such as immunocompetent cells and vascular endothelial cells (Regnier et al., 1997; Guironnet et al., 2001; Ponec, 2002; Supp et al., 2002), might also provide insight into biological and pharmacological responses.

## Dermal and skin equivalents for skin replacement

Several groups have employed skin equivalents for wound management for acute and chronic wounds (Boyce, 1996; Singer and Clark, 1999; Coulomb and Dubertret, 2002). Boyce and colleagues (Boyce, 1996; Boyce and Warden, 2002; Boyce et al., 2002) used skin equivalents prepared from autologous human keratinocytes and fibroblasts for grafting onto wound sites and found that these grafts are equivalent to autologous split-thickness skin grafts. Furthermore, the requirement for harvesting donor skin was less than that for conventional skin autografts. Inclusion of a 'dermal' component provides an environment that promotes vascularization of the graft, and fibroblasts play an active role in the replacement of the dermal matrix (Demarchez et al., 1992; Supp et al., 2002).

## Concluding remarks

Fibroblasts represent a diverse population of cells (Fries et al., 1994). Phenotypic differences are manifested in a variety of ways: extracellular matrix production and organization, production of growth factors/cytokines, and participations in inflammatory responses (Fries et al., 1994; Smith et al., 1997; Doane and Birk, 1991; Limeback et al., 1982; Derdak et al., 1992; Stephens et al., 2001). In the skin, two forms of fibroblast heterogeneity have been noted. Intrasite heterogeneity relates to the position of fibroblasts in the context of epidermal structures. Thus, papillary, reticular and hair-follicle-associated fibroblasts differ from each other. A second type of heterogeneity is based upon the anatomical location within the body. Thus, interfollicular fibroblasts from scalp, face, trunk, leg, and so on exhibit subtle differences from each other. Less is currently known about these intersite differences in fibroblasts. Chang et al. have shown that human dermal fibroblasts obtained from various anatomical sites express different homeobox transcription factors (Chang et al., 2002). The AP-1 family of transcription factors is important in regulating the production of factors that regulate epithelial-mesenchymal interactions, cellular proliferation and extracellular matrix production (Angel and Szabowski, 2002; Shaulian and Karin, 2002). Papillary and reticular dermal fibroblasts differ in these characteristics. Therefore, additional studies related to this family of factors might help us to understand the differences between subpopulations of dermal fibroblasts.

Fibroblast diversity in the skin raises questions that will require experiments to provide answers. Inductive influences from the epidermis result in the differentiation of fibroblasts associated with hair follicles. However, the factor(s) or event(s) that drives the differentiation of papillary and reticular cells are unknown. Furthermore, our knowledge of the physiological characteristics that differentiate papillary from reticular fibroblasts remains limited. Additional information in this regard will expand our conceptualization of the function of fibroblasts in skin. There is currently limited information that suggests that *AP-1* and *homeobox* genes and their regulators play roles in determining fibroblast diversity. Additional studies are required to define the roles of these and possibly other regulatory genes in establishing and maintaining fibroblast diversity. With the increased reliance on the development and application of three-dimensional skin equivalents for biological and clinical purposes, it will be necessary to be more selective about the choice of fibroblast to be employed.

Finally, the term 'dermal fibroblast' is an oversimplification. In reality, dermal fibroblasts are a dynamic, diverse population of cells. This means that we should take greater care defining the population of dermal fibroblast that is used in experimental studies. We are only beginning to understand the function of these cells in defining the structure and organization of skin and their complex intercellular interactions. Our current knowledge of fibroblast physiology is largely based upon monolayer culture studies. These studies more closely reflect the status of these cells in an early wound repair situation. The use of three-dimensional dermal and skin equivalents in future studies should provide more relevant information regarding possible physiological differences between fibroblast subpopulations in vivo. Much work will be required in the future if we are to understand and appreciate fully this diverse population of cells.

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