European Journal of Cell Biology 94 (2015) 483–512

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Review

Fibroblast heterogeneity and its implications for engineering organotypic skin models *in vitro*

Gopu Sriram[a,1], Paul Lorenz Bigliardi[a,b,c], Mei Bigliardi-Qi[a,*]

[a] *Experimental Dermatology Laboratory, Institute of Medical Biology, Agency for Science, Technology and Research, Singapore, Singapore*
[b] *National University of Singapore NUS, YLL School of Medicine, Singapore, Singapore*
[c] *Division of Rheumatology, University Medicine Cluster, National University Hospital, Singapore, Singapore*

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 11 August 2015
Accepted 11 August 2015

Keywords:
Organotypic skin
Fibroblast heterogeneity
Wound healing
Skin models
Skin substitutes
Tissue engineering

ABSTRACT

Advances in cell culture methods, multidisciplinary research, clinical need to replace lost skin tissues and regulatory need to replace animal models with alternative test methods has led to development of three dimensional models of human skin. In general, these *in vitro* models of skin consist of keratinocytes cultured over fibroblast-populated dermal matrices. Accumulating evidences indicate that mesenchyme-derived signals are essential for epidermal morphogenesis, homeostasis and differentiation. Various studies show that fibroblasts isolated from different tissues in the body are dynamic in nature and are morphologically and functionally heterogeneous subpopulations. Further, these differences seem to be dictated by the local biological and physical microenvironment the fibroblasts reside resulting in "positional identity or memory". Furthermore, the heterogeneity among the fibroblasts play a critical role in scarless wound healing and complete restoration of native tissue architecture in fetus and oral mucosa; and excessive scar formation in diseased states like keloids and hypertrophic scars. In this review, we summarize current concepts about the heterogeneity among fibroblasts and their role in various wound healing environments. Further, we contemplate how the insights on fibroblast heterogeneity could be applied for the development of next generation organotypic skin models.

Contents

Abbreviations: 3D, three-dimensional; AdSCs, adipose-derived stem cells; AP-1, activated protein-1; Blimp1, B lymphocyte-induced maturation protein-1; BP, bullous pemphigoid antigen; CD, cluster of differentiation; Dlk1, delta-like 1 homolog; DOPr, Delta-opioid receptor; DS, dermal sheath; ECM, extracellular matrix; En1, Engrailed-1; FDP, follicular dermal papilla; FGF, Fibroblast growth factor; FSP-1, fibroblast-specific protein-1; GM-CSF, granulocyte-macrophage colony stimulating factor; HA, hyaluronic acid; HAS, hyaluronan synthase; hESCs, human embryonic stem cells; HGF, hepatocyte growth factor; IL, interleukin; iPSCs, induced pluripotent stem cells; KGF, keratinocyte growth factor; KOPr, kappa-opioid receptor; Lrig1, leucine-rich repeats and immunoglobulin-like domains 1; mAChR, mucarinergic acetyl choline receptors; MF, mitotically active fibroblasts; MMP, matrix metalloproteinases; MOPr, Mu-opioid receptor; Myb, myeloblastosis viral oncogene; nAChR, nicotinergic acetyl choline receptors; PDGFR, platelet-derived growth factor receptors; PMF, post-mitotic fibrocytes; PPAR, peroxisome proliferative-related receptors; RAR, retinoic acid receptors; RXR, retinoid X receptors; TGFβ, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor; α-SMA, α-smooth muscle actin.

* Corresponding author at: Experimental Dermatology Laboratory, Institute of Medical Biology, Agency for Science, Technology and Research A*STAR, Singapore 138648, Singapore. Tel.: +6591371809.
E-mail addresses: sriram.gopu@imb.a-star.edu.sg (G. Sriram), paul.bigliardi@gmail.com (P.L. Bigliardi), mei.bigliardi@gmail.com (M. Bigliardi-Qi).
[1] http://www.orcid.org/0000-0001-8423-5197.

http://dx.doi.org/10.1016/j.ejcb.2015.08.001

1. Introduction

Skin is the largest organ in the human body that consists of tightly organized layers of keratinocytes and the underlying dermis. The skin is highly specialized in terms of providing barrier function by preventing water loss, resisting mechanical trauma, protection against physical, chemical and/or biological agents such as temperature, light, metals, chemicals, toxins, micro-organisms (fungi, bacteria, viruses) (Simpson et al., 2011). In addition to the barrier function, the skin plays a role in sensation, thermoregulation, excretion, absorption, pigmentation and innate/acquired immunity. In addition, the skin has very complex appendages, such as hair follicles, sebaceous glands, sweat glands and nails. Recapitulating the skin with such complex functionalities *in-vitro* is a daunting task that requires the need for a complex three-dimensional (3D) microenvironment that cannot be provided by conventional monolayer cultures. Advances in cell culture methods, multidisciplinary research, clinical need to replace lost skin tissues and regulatory need to replace animal models with alternative test methods has led to development of 3D organotypic models of human skin consisting primarily of keratinocytes over fibroblasts-populated dermal matrices. The need for these organotypic models of skin are immense and have profound implications not only for basic scientists and tissue engineers, but also for clinicians, manufacturers, regulatory authorities and animal welfare organizations. Thus it is imperative that we understand how normal skin develops, is regulated, and heals during recovery from wounding. What we now know is that fibroblasts are an integral part of mesenchyme-derived signals that are essential for epidermal morphogenesis, homeostasis and differentiation.

Fibroblasts are the most abundant cell type within all the body's connective tissues, and their primary role is secretion of the components of the extracellular matrix (ECM). In the case of the skin, the epidermal keratinocyte layer is underpinned and intimately connected to the dermis, which contains the dermal fibroblasts as well as immune cells, blood vessels, nerve fibers, hair follicles and secretory glands. Early attempts to model the human skin *in vitro* used monolayer cultures of keratinocytes and/or fibroblasts, and while these studies formed the foundation of our current knowledge, they were unsuitable for studying the interaction between the two cell types as they were unable to take into account the effect of spatial organization of the skin layers. In addition, they do not have a corneal layer and a differentiated epidermis with the variety of properties of the keratinocytes in different differentiation stages. Advances in culturing techniques have since led to the development of organotypic culture systems that mimic the 3D organization of keratinocytes and fibroblasts observed *in vivo* (Auxenfans et al., 2009). Keratinocytes are seeded onto fibroblast-populated dermal matrices then cultured at the air–liquid interface to drive epidermal differentiation, stratification and cornification, which results in an engineered skin tissue that closely mimics the native skin (Bell et al., 1981; Boehnke et al., 2007; Butler et al., 2008; Cario-Andre et al., 2006; Chen et al., 1995; El Ghalbzouri et al., 2002a,b, 2005; Lamb and Ambler, 2013; Liu et al., 2007; Muffler et al., 2008; Ponec et al., 1997, 2001; Pontiggia et al., 2013; Smola et al., 1994; Stark et al., 1999, 2004a,b, 2006). These engineered skin mimics have successfully been used to study various aspects of skin biology including epithelial-mesenchymal interactions (Maas-Szabowski et al., 1999; Smola et al., 1993, 1994), growth and differentiation of keratinocytes (Boukamp et al., 1990; Maas-Szabowski et al., 2000; Muffler et al., 2008; Stark et al., 2004b, 2006), development of epithelial barrier properties (Pasonen-Seppanen et al., 2001; Ponec et al., 1997, 2001; Regnier et al., 1993; Thakoersing et al., 2012), dynamics of the basement membrane (Breitkreutz et al., 1997, 2004; El Ghalbzouri et al., 2005; Fleischmajer et al., 1998; Nischt et al., 2007; Smola et al., 1998; Stark et al., 2004b), wound healing (Boyce and Warden, 2002; Geer et al., 2002; Harrison et al., 2006; Laplante et al., 2001) and dermatopathology (Barker et al., 2004; Butler et al., 2008; Chiu et al., 2005; Eves et al., 2000). They have also revealed new and intriguing properties of the dermal fibroblast population with profound implications for our understanding of how the skin works and how we might be able to engineer optimal skin substitutes for industrial and clinical use.

In this review we will discuss current knowledge and recent breakthroughs in our understanding of fibroblasts, with particular reference to the dermal fibroblast population and its effects on human skin development, homeostasis and healing. We will look at how knowledge gained from *in vivo* wound healing studies and scarring phenotypes can be combined with the latest insights from reconstituted skin models to inform the next steps in development of biological skin substitutes. Finally, we will highlight several questions in the field and consider how the next generation of *in vitro* models might be optimized to find the answers and facilitate development of clinically-appropriate skin replacements.

2. Fibroblasts in the skin

Conventionally, fibroblasts are defined by their spindle-shaped morphology, adhesive growth on tissue culture plastics, expression of mesenchymal markers that include vimentin and collagen I, and the lack of expression of markers related to other specific cell lineages. While traditionally considered a static population of spindle-shaped cells that maintain and support the skin through secretion and degradation of ECM, we now know that fibroblasts play an important role in almost every skin process throughout life: in the embryo, fibroblasts direct skin morphogenesis; in the mature organism they contribute to homeostasis of the skin; and their involvement in various physiopathological conditions including healing, fibrosis, aging, psoriasis, and skin cancer, is just beginning to be understood. The dermal fibroblast population also undertakes dynamic and reciprocal interactions with other resident cell types (epithelial cells, endothelial cells, neural cells, adipocytes, inflammatory cells, and resident stem cells) through direct cell–cell communications, cell–matrix interactions, and the secretion of soluble factors (growth factors and cytokines) (Borchers et al., 1994; Costea et al., 2003; Eming et al., 2007; Maas-Szabowski et al., 1999;

Mueller and Fusenig, 2002; Werner et al., 1992, 2007; Werner and Smola, 2001).

Recent studies have also shown that the fibroblasts of the skin exist as morphologically- and functionally- heterogeneous subpopulations: the fibroblast population within the superficial layers of the dermis (the papillary dermis) is physiologically distinct from that residing in deeper layers (the reticular dermis), and different again is the population associated with hair follicles (follicular dermal papilla (FDP) and dermal sheath (DS) fibroblasts) (Chuong et al., 2007; Harper and Grove, 1979; Honardoust et al., 2012a,b; Jahoda and Reynolds, 2001; Ohyama et al., 2010; Schafer et al., 1985; Sorrell et al., 2004, 2008). Advances in molecular techniques have begun to shed light on the precise nature of fibroblast heterogeneity, revealing that despite their common phenotype, dermal fibroblast subpopulations exhibit distinct gene expression patterns and variable functions (Chang et al., 2002; Fries et al., 1994; Rinn et al., 2006).

2.1. Fibroblast diversity

The fibroblast population of the adult body exhibits functional diversity that is generated on several levels, beginning with the embryonic origin of the population, then by the tissue and microenvironment of residence.

2.1.1. The embryonic origin of fibroblasts

Fibroblasts in different parts of the body arise from different embryonic origins; while those in the face are generated from the neural crest, dorsal skin from the dermato-myotome, and ventral skin comes from the lateral plate mesoderm (Houzelstein et al., 2000; Le Lievre and Le Douarin, 1975; Ohtola et al., 2008; Wong et al., 2006) (Fig. 1). Recently, two elegant studies in mice have identified distinct population of dermal fibroblasts during development and in the adult skin. Using lineage tracing studies in mice, Driskell et al. (2013) identified that dermal fibroblasts arise from a multipotent progenitor population that expresses *PDGFRα*, *Dlk1* and *Lrig1*; which upon differentiation gives rise to all the dermal fibroblast lineages. During early development, *PDGFRα*, *Lrig1*, and *Blimp1* expressing subpopulations give rise to fibroblasts of the papillary dermis; while *PDGFRα*, *Dlk1* and *Sca1* expressing subpopulation gives rise to reticular and hypodermal fibroblast lineages. Further differentiation and fate restriction of these subpopulations give rise to the different fibroblast subsets of the papillary, reticular and hypodermis. Similarly, Rinkevich et al. identified the existence of two distinct embryonic fibroblast (*Engrailed-1* (*En1*)-positive and negative) lineages, the embryonic expression of *En1* being responsible for the deposition of the bulk of dermal connective tissue during embryonic development, cutaneous wound healing, radiation-associated fibrosis and cancer stroma (Rinkevich et al., 2015). Further they demonstrated that the fibrogenic fibroblast lineage could be identified by its expression of CD26/DPP4; and small molecule-based inhibition of DPP4 enzymatic activity during wound healing was associated with diminished scarring. However, the relevance of these findings to development of human skin needs to be validated.

Alongside the embryonic origin of developing fibroblast populations, their spatial organization within the embryo drives pattern formation, gives important positional information, and ultimately results in formation of tissues and organ systems. These spatial patterns and positional information underlies cellular specialization and therefore has to be maintained throughout the life span of the living being as the tissues undergo renewal, repair and regeneration. This is well-illustrated in the skin: selective distribution of body hair and sweat glands is a classic example of spatial specialization; similarly, many dermatological conditions (for instance, acne and keloids) are much more likely to occur at specific anatomic sites. Intriguingly, it appears that much of the spatial specialization seen in the skin is in fact driven by the underlying dermal fibroblasts. Early heterotopic recombination studies using dermal and epidermal explants first suggested the deterministic role of dermal tissues upon the epidermis, in particular in defining positional identity during embryogenesis (Malacinski and Bryant, 1984). This was initially indicated by experiments where dental epithelium derived from chick embryos was cultured upon dermal mesenchyme which drove development of a complete skin epidermis with appendages; accordingly, dental mesenchyme directed the differentiation of skin epidermis towards formation of a dental organ (Kollar, 1986). Similarly, recombination of wing epithelium with leg mesenchyme results in the generation of scales instead of feathers (Dhouailly et al., 1978). Extending these observations using *in vitro* skin equivalent models found that human dermal fibroblasts derived from palmoplantar sites induce keratinocytes of non-palmoplantar origin to form an epidermis with hyperkeratosis and keratin-9 expression reminiscent of an *in vivo* palmoplantar epidermal phenotype (Yamaguchi et al., 1999). Taken together, such heterotopic recombinant studies elegantly reveal the instructive role of mesenchymal tissues towards their overlying epithelia, which is crucial during skin development, repair and disease.

2.1.2. Anatomic heterogeneity among fibroblasts

Even within fibroblast populations that share a common embryonic origin considerable diversity exists as a product of distinct anatomic locations and their associated microenvironments. As early as the 1960's, it was known that fibroblasts derived from skin, mesothelium, joints or periosteum exhibited detectable differences in metabolic activity (Castor et al., 1962). In the case of the skin, dermal papillary fibroblasts from hair-bearing areas interact with follicular epithelial cells to induce hair formation, while dermis from the glabrous skin of palms and soles instructs for alternative epithelial fates (Kishimoto et al., 1999). Moreover, such fibroblasts derived from glabrous skin are smaller, are more proliferative, and express lower levels of transforming growth factor-β1 (TGFβ1)-receptor II, α-smooth muscle actin (α-SMA) and fibronectin compared to fibroblasts from non-glabrous skin (Chipev and Simon, 2002).

Molecular interrogation of fibroblast populations from various anatomic sites has added another dimension to our appreciation of fibroblast heterogeneity and complexity. Genome-wide gene expression profiling studies of primary fibroblasts from different anatomical sites in fetal and adult humans have uncovered site-specific differences in gene expression profiles relating to patterning and positional information based on anterior-posterior, proximal-distal and dermal-nondermal anatomic divisions (Chang et al., 2002; Rinn et al., 2006). These studies found marked topographic differences in expression of genes related to growth and differentiation, ECM production, cell migration, lipid metabolism and various genodermatoses. Interestingly, it now seems that topographic heterogeneity is manifested in the context of retained embryonic gene expression patterns; comparison of fetal and adult human fibroblasts revealed ongoing expression of key HOX genes, which direct the development of the embryonic body plan, in adult fibroblasts (Rinn et al., 2006), indicating that the HOX family of transcription factors may continue to provide positional information throughout the adult life of fibroblasts. Further studies will be required to link the observed differences in gene expression between various fibroblast sub-populations and the underlying mechanisms that determine fibroblast phenotype and function. Understanding the molecular regulation of fibroblasts between and within specific anatomic sites will open the door for development of tailored skin substitutes both for research and clinical use in patients requiring reconstruction in those regions.

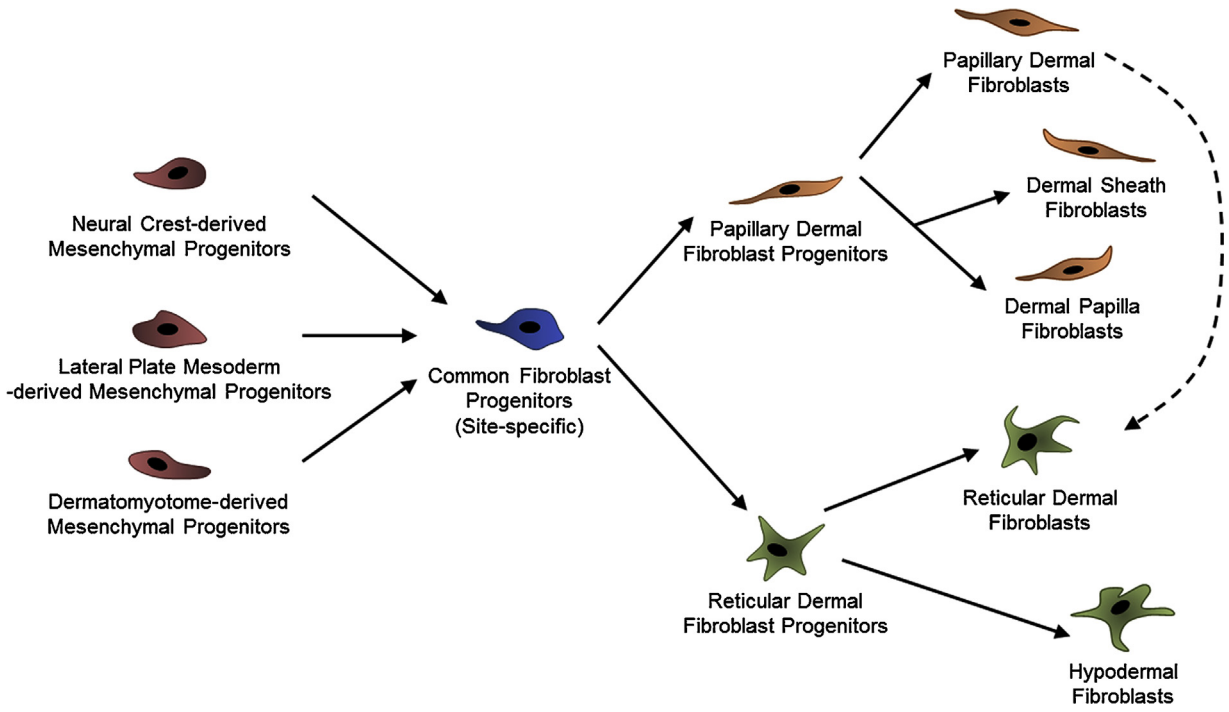

Fig. 1. Schematic model depicting the developmental heterogeneity in the origin of dermal fibroblasts based on studies in humans and mice. Fibroblast progenitors arise from different developmental origins like neural crest, lateral plate mesoderm and dermatomyotome depending on the site of skin. These site-specific fibroblast progenitors undergo lineage commitment to papillary and dermal fibroblast progenitors that further differentiates to give rise to different fibroblast populations within the skin. Evidences also suggest that papillary dermal fibroblasts upon aging might differentiate to reticular dermal fibroblast phenotype.

2.1.3. Heterogeneity within the dermal fibroblast compartment

Besides the differences among fibroblasts from different parts of the body, the fibroblasts within a single tissue like the skin are likewise not composed of a homogeneous population. Studies suggest the existence of anatomic subsets of dermal fibroblasts that are defined by their location within the dermis: fibroblasts that exist within the papillary dermis, the reticular dermis, and in association with hair follicles (FDP and DS fibroblasts) (Cormack, 1987; Sorrell and Caplan, 2004) (Fig. 2). Papillary dermis is the superficial portion of the dermis that lies immediately beneath the epidermis and contains the rete ridges, those projections of the epidermis that serve to increase the surface area for interactions between the epidermal and dermal layers. The papillary dermis is demarcated from the underlying reticular dermis by a vascular plexus, the rete subpapillare: between this superficial vascular plexus and the deep vascular plexus (rete cutaneum) is the reticular dermis, and below rests the hypodermis. Hair follicles and their associated dermal cells are most often found in the hypodermis, while the papillary dermis is the thinnest, most vascular and most innervated region within the dermis.

Various studies have demonstrated that papillary and reticular dermal fibroblasts are morphologically and physiologically distinct and the ECMs these fibroblasts reside also exhibit differences in terms of their composition and organization (Azzarone and Macieira-Coelho, 1982; Schafer et al., 1985; Schönherr et al., 1993; Sorrell et al., 2004). Table 1 highlights the differences between papillary dermis and reticular dermis (human) observed *in vivo* and through *in vitro* studies. Papillary dermis is composed of thin, poorly-organized collagen fiber bundles while thick, well-organized collagen bundles characterize the reticular dermis (Sorrell and Caplan, 2004). The papillary dermis also has a higher ratio of collagen type-III to I, higher levels of decorin, and lower levels of the chondroitin proteoglycan versican, compared to the reticular dermis (Schönherr et al., 1993; Sorrell et al., 1999, 2004; Zimmermann et al., 1994). Similarly, other ECM molecules are also differentially apportioned between the two dermal compartments. Such differences in the ECM composition and organization play a role in controlling the fibroblast behavior and their response in wound healing and scar formation. In addition, to differences in the ECM, *in vitro* studies using papillary and reticular dermal human fibroblasts demonstrate that these differences between the dermal layers are reflected at the cellular level (Table 1). Papillary dermal fibroblasts display higher growth kinetics and lower contractile properties *in vitro* compared to site-matched reticular fibroblasts (Azzarone and Macieira-Coelho, 1982; Harper and Grove, 1979; Schafer et al., 1985; Sorrell et al., 2004). Papillary dermal fibroblasts synthesize more decorin, while versican is produced at higher levels by reticular dermal fibroblasts both *in vivo* and *in vitro* (Schönherr et al., 1993). While Tajima and Pinnell found no differences between the two fibroblast populations in terms of synthesis of collagen types-I and III *in vitro*, they did observe that media conditioned by papillary dermal fibroblasts had higher amounts of type I procollagen (Tajima and Pinnell, 1981). Furthermore, Akagi et al. (1999) found higher expression of collagen type XVI transcripts in fibroblasts derived from the upper layers of the dermis compared to middle and deeper layers, which correlated with preferential immunohistochemical labeling for collagen type XVI in the upper dermis of normal human skin. Collagen type XVI is a member of the fibril associated collagens and constitutes a minor component of the skin ECM. However, its presence in the dermo-epidermal zone of the papillary dermis is associated with an active role in anchoring microfibrils to basement membranes and hence ensures mechanical anchorage of the basal keratinocytes to the basement membrane (Grassel et al., 1999; Kassner et al., 2003). However, in scleroderma the levels of collagen type XVI are upregulated and it is expressed in both papillary and reticular dermis (Akagi et al., 1999). On the other hand, human dermal fibroblast senescence *in vitro* is associated with decrease in expression of integrin-α5 and collagen type XVI resulting in reduced cellular adhesion (Mancini et al., 2012). Overall, these studies indicate the

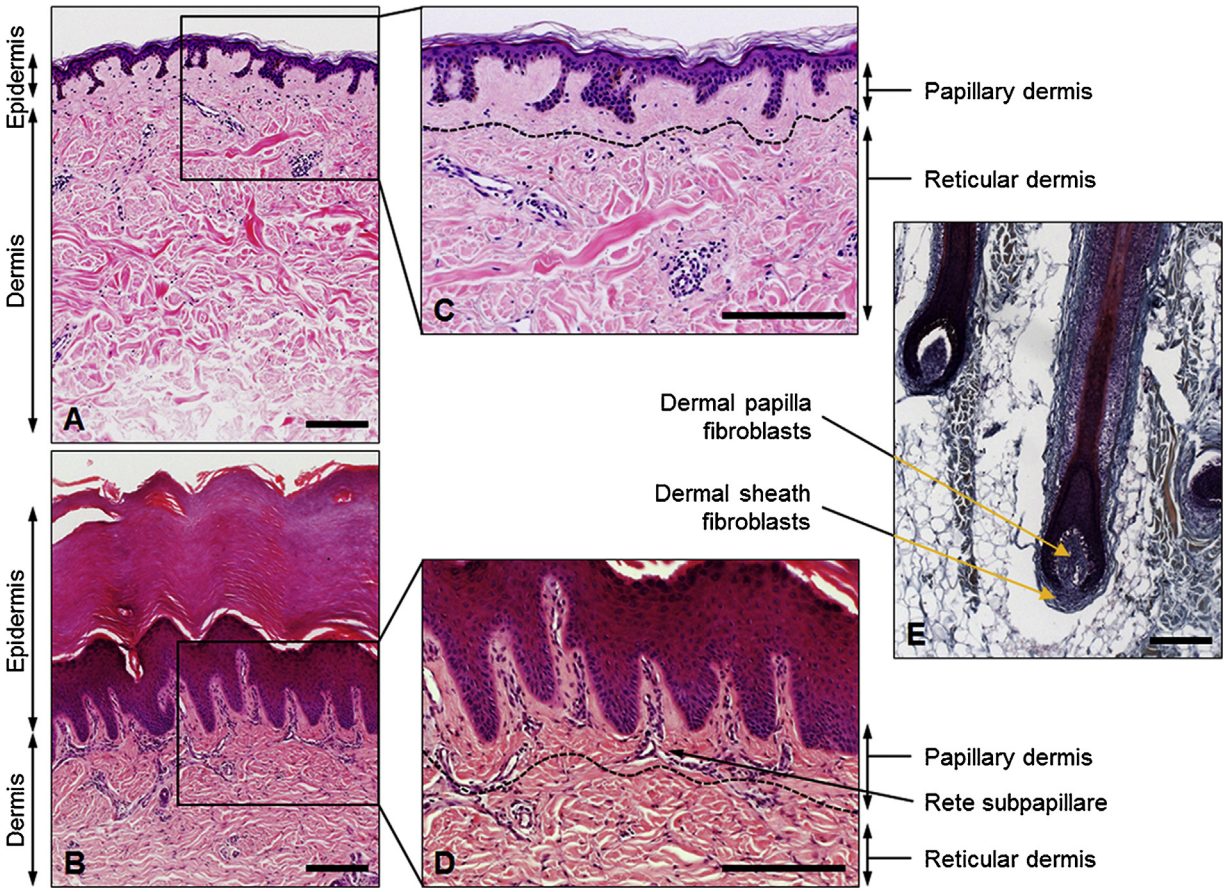

Fig. 2. Adult human skin consists of epidermis and dermis. The epidermis is thrown into folds called rete ridges (A and B). Depending on the region of the body the epidermis and its cornified layer various in thickness. For instance, the skin on the back is thin (A), while the skin on the palms is made of thick epidermis and thick stratum corneum (B). The dermis is divided into papillary and reticular dermis (C and D) that are separated by a vascular plexus termed the rete subpapillare (D). The papillary dermis contains higher density of cells and fine collagen bundles compared to reticular dermis that is less cellular and is composed of thick and organized collagen bundles. The arbitrary dashed line in (C and D) is drawn based on the above-mentioned differences in organization of collagen fibers and location of rete subpapillare. This demarcation between papillary and reticular dermis could be subjective and might be difficult in skin from older individuals. The papillary and reticular dermis consists of two distinct populations of dermal fibroblasts. In addition to these two populations, the skin also has fibroblasts associated with dermal papilla and dermal sheath of the hair follicle (E) which are usually located in deeper reticular dermis and hypodermis. Scale bar: 200 μm. (B, D and E: kindly provided by Declan Lunny, Institute of Medical Biology, Singapore).

contribution of papillary and reticular dermal fibroblasts towards differences in the ECM composition within the two compartments of the dermis in health and diseased states.

Alongside their differences in ECM synthesis, the papillary and reticular dermal fibroblast populations exhibit distinct patterns of growth factor and cytokine secretion that have profound implications for their paracrine cross-talk with neighboring keratinocytes. Cultured papillary dermal fibroblasts produce a higher ratio of granulocyte-macrophage colony stimulating factor (GM-CSF) to keratinocyte growth factor (KGF) compared to site-matched reticular dermal fibroblasts (Sorrell et al., 2004). Similarly, papillary dermal fibroblasts express higher levels of interleukin (IL)-6 *in vitro* both constitutively and upon exposure to IL-1α (Sorrell et al., 2004). Compared to superficial dermal fibroblasts, those cultured from deeper layers of the dermis express more TGFβ, connective tissue growth factor, α-SMA, heat shock protein 47, type I collagen and versican, a phenotype associated with hypertrophic scarring (Varkey et al., 2011; Wang et al., 2008). Global gene expression profiling has revealed that cultured human reticular fibroblasts overexpress genes involved in cell motility, cytoskeletal organization, smooth muscle contraction pathway like α-SMA, and neuronal development (Janson et al., 2012). On the other hand, the cultured papillary dermal fibroblasts express high levels of genes related to the complement activation pathway implying its role in skin immune response (Janson et al., 2012). Thus, despite their close physical proximity, fibroblast populations from the papillary and reticular dermis are readily distinguished by their phenotypic and functional features, reflecting the distinct physiological niches they occupy within the skin.

In addition to papillary and reticular fibroblasts, a third clearly demarcated niche within the skin is occupied by a subpopulation of dermal fibroblasts that are associated with hair follicles (Fig. 2). Hair follicles are associated with two fibroblast subpopulations: FDP fibroblasts and DS fibroblasts. Dermal papillae of the hair follicle consist of a small cluster of densely packed fibroblasts that are located within the bulb region of the hair follicle (Chuong et al., 2007). These follicular dermal papilla fibroblasts (FDP fibroblasts) through interactions with the epithelial cells of the hair follicle, play a primary role in determination of hair bulb size, length and diameter of the hair shaft and the duration of anagen phase in the hair cycle (Schneider et al., 2009). DS fibroblasts are the fibroblasts present within the loose connective tissue sheath that surrounds the entire hair follicle (Ohyama et al., 2010). They are separated from the epithelial outer root sheath of the hair follicle by a specialized basement membrane called the glassy membrane (Jahoda and Reynolds, 2001).

FDP and DS fibroblasts from human hair follicles differ from other dermal fibroblasts by their higher expression levels of non-specific alkaline phosphatase, α-SMA, and protease-activated receptor-1 *in vivo* (Anan et al., 2003; Ohyama et al., 2010).

Table 1
Differences among fibroblasts and the surrounding microenvironment within papillary and reticular dermal layers of the skin.

	Papillary dermis	Reticular dermis
In-vivo		
Collagen type I: type III (ratio)	Low	High
Collagen type IV	Positive (basement membrane)	Negative
Collagen VI	Positive (BMZ/DEJ)	Weakly positive
Collagen XII	Positive	Low to Absent
Collagen XIV	Low to absent	Positive
Collagen XVI	Positive (BMZ/DEJ)	Negative
α-SMA	Negative	Predominantly negative (except positive staining in dermal sheath and around blood vessels)
Tenascin-C	Positive (BMZ/DEJ)	Negative
Tenascin-X	Weakly Positive (BMZ/DEJ)	Positive
Versican	Diffusely weak-positive (associated with microfibrils)	Positive (along with elastic fibres)
Decorin	Positive	Weak positive
Elastic fibers	Thin, oriented perpendicular to DEJ	Coarse, parallel to DEJ
MGP	Weak to Negative	Positive
Fibronectin	Positive (DEJ and blood vessels)	Positive (associated with blood vessels)
Hyaluronan	Positive	Diffuse
In-vitro		
Size and Morphology	Spindle-shaped and thin	Spindle to polygonal; thick
Proliferation	Fast	Slow
Vimentin	Positive	Positive
Versican	Negative	Positive
Podoplanin	Positive	Negative
Calponin	Negative	Positive
Transglutaminase	Negative	Positive
α-SMA	Negative	Positive/Negative
NTN-1	Positive	Negative
KGF	Lower	Higher
VEGF	Higher	Lower
TGF-β1	Lower	Higher
MMPs	Higher	Lower
TIMPs	Higher	Lower

α-SMA—α-smooth muscle actin; BMZ—basement membrane zone; DEJ—dermo-epidermal junction; KGF—keratinocyte growth factor; MGP—matrix Gla protein; MMPs—matrix metalloproteinases; NTN-1—netrin-1; TIMPs—tissue inhibitor of metalloproteinases; VEGF—vascular endothelial growth factor.

However, the expression of various markers in FDP and DS fibroblasts vary depending on species, stage of hair cycle, and whether they are analyzed on histological samples or using *in vitro* cultured cells. In humans, α-SMA is one of the markers that differentiates DS fibroblasts from FDP and other dermal fibroblasts (α-SMA is expressed only in DS but not in FDP) (Jahoda et al., 1991). However, upon *in vitro* culture the FDP fibroblasts become α-SMA positive (Jahoda et al., 1991). Hence, α-SMA could be used as a marker for DS fibroblasts *in vivo*, and as a marker for both FDP and DS fibroblasts *in vitro*. Similarly, in human hair follicles (anagen), versican is expressed strongly in FDP, while the DS exhibits weak immunoreactivity. However, its expression in FDP is lost in miniaturized hair follicles afflicted by androgenetic alopecia (Soma et al., 2005), and seems to be upregulated by induction with ascorbic acid 2-phosphate (Kim et al., 2006). Though both FDP and DS fibroblasts are specialized fibroblasts of mesenchymal origin, the expression of neuronal markers by FDP fibroblasts, their transcriptional profile and lineage tracing studies in mice suggests a neural crest origin (Driskell et al., 2009, 2013; Fernandes et al., 2004; Rendl et al., 2005). Other markers that are reported to be expressed in mouse/human FDP fibroblasts include perlecan, syndecan-1, CD133, endothelin-1, nexin-1, stem cell factor and CD44 (Couchman, 1986; Ohyama et al., 2010; Sorrell and Caplan, 2009). Studies have also shown that human hair follicle associated fibroblasts express higher amounts of growth factors like stem cell factor and TGFβ2 (Chiu et al., 1996; Hibberts et al., 1996); and lower amounts of matrix metalloproteinases (MMP-3 and MMP-9) (de Almeida et al., 2005) compared to the interstitial dermal fibroblasts *in vitro*. Studies have indicated the presence of heterogeneity among FDP fibroblasts from different parts of the body. For instance, certain subsets of FDP fibroblasts, especially those in male facial skin, express androgen receptors (Itami et al., 1995), while those in the skin of the upper legs and buttocks of females express estrogen receptors (Pugliese, 2007). Further, studies on FDP fibroblasts from balding and non-balding regions of the scalp in androgenetic alopecia revealed differences in terms of growth characteristics, expression of androgen receptors, and senescence (Bahta et al., 2008; Hibberts et al., 1998; Moon et al., 2013; Randall et al., 1996). These studies indicate that the fibroblast populations associated with the hair follicles have important roles in epidermal morphogenesis, hair morphogenesis, and in regulating abnormal hair loss.

For many years it has been believed that the dermis contains well-defined fibroblast subpopulations. It should be noted that studies show the presence of additional subtypes within the intermediate zones of the dermis (Akagi et al., 1999; Ali-Bahar et al., 2004), but these populations remain incompletely characterized and hence, the true extent of dermal fibroblast heterogeneity is not fully understood. While much remains to be studied, it has long been known that the fibroblast populations of a given site can be subdivided by differences in morphology, replicative potential and biochemical activity, which was thought to relate to their position in an ongoing ageing process (Rodemann et al., 1989a). Fibroblasts were found to exist as either 'mitotically active' or 'replicative progenitor' fibroblasts (MF) and 'irreversible post-mitotic' fibrocytes (PMF), which represent a senescent synthetic phenotype (Bayreuther et al., 1988a,b; Rodemann et al., 1989a). This led to a system of classification where fibroblasts were subdivided into MF and PMF phenotypes (Rodemann et al., 1989a). Besides differences in their proliferative capacities *in vitro*, PMFs also produced 5–8 times more total collagen than did MFs which is thought to be important for maintaining homeostatic levels of interstitial collagen types I, III and V (Rodemann et al., 1989a). Studies have shown that the ratio of MFs to PMFs seems to be important for the biochemical homeostasis of dermal tissues and in pathologic tissue remodeling (Bayreuther et al., 1988a; Herskind et al., 1998; Rodemann et al., 1989b). More recent work has confirmed and extended this model: clonal studies using cells cultured from the superficial layer of the dermis detected some rapidly proliferating cells that are smaller and do not express α-SMA, and a second population of larger, slower proliferating cells that are positive for α-SMA and produced rapid contraction of collagen gels (Sorrell et al., 2007).

The impact of these observations on fibroblast phenotypes extends beyond the dermal compartment and into their interactions with keratinocytes in the epithelium. Fibroblasts communicate with keratinocytes both through direct cell–cell interactions and paracrine factors including IL6, IL8, KGF, hepatocyte growth factor (HGF) and TGFβ1 (Boukamp et al., 1990; Maas-Szabowski et al., 1999; Werner and Smola, 2001; Werner et al., 1994; Xia et al., 2004). Studies using cocultures of keratinocytes and fibroblasts have demonstrated that the keratinocytes instruct the fibroblasts to produce growth factors and cytokines like KGF (FGF-7), IL-6, FGF-10 and GM-CSF. IL-1 has been identified as one of the primary inducers secreted by keratinocytes (Braun et al., 2004; Maas-Szabowski and Fusenig, 1996; Maas-Szabowski et al.,

1999; Steiling and Werner, 2003; Werner et al., 1992, 1994). Monolayer and organotypic coculture studies have also revealed that keratinocyte-derived IL-1 regulates the expression of KGF, GM-CSF, pleiotrophin and stromal-cell derived factor-1 in dermal fibroblasts through transcription factor activated protein-1 (AP-1) (Werner, 1998; Werner and Grose, 2003; Werner et al., 1992; Werner and Smola, 2001). These paracrine factors secreted by dermal fibroblasts in turn regulate keratinocyte proliferation and differentiation in cocultures *in vitro* (Florin et al., 2005). Similarly, a recent study in mice has demonstrated that targeted deletion of avian myeloblastosis viral oncogene (Myb) in $K14^+$ keratinocytes was associated with defects in epithelial proliferation, thickness and morphology (Sampurno et al., 2015). In addition to the effects on epidermis, deletion of Myb in keratinocytes was associated with deficient production of collagen type-I by fibroblasts, which seems to be due to a reduced expression of TGFβ1 by the keratinocytes. All these observations underline the concept of double paracrine signaling between keratinocytes and fibroblasts, wherein the former initiates growth factor production in fibroblasts, which in turn stimulates keratinocyte proliferation and differentiation. The primary function of fibroblast-derived paracrine factors is to drive keratinocyte proliferation and migration, with the exception of TGFβ1, that down-regulates proliferation and induces keratinocyte differentiation. Studies using fibroblasts treated with mitomycin-C to mimic the PMF phenotype revealed significantly higher secretion of KGF *in vitro* compared to MF subtypes, and among the MF subtypes, MF-III produced more KGF than MF-II, with MF-I producing the least (Nolte et al., 2008). In parallel, TGFβ1 is predominantly secreted by MF-III and PMFs (Nolte et al., 2008). Studies on monolayer and organotypic keratinocyte-fibroblast cocultures have also demonstrated that these differences in KGF secretion are mediated by AP1 complex (Maas-Szabowski et al., 2000, 2001; Szabowski et al., 2000). Hence, *via* a double paracrine signaling, keratinocytes and the sub-populations of fibroblasts combine to fine tune the balance between keratinocyte proliferation, migration and differentiation, as well as influence the ECM synthesis and remodeling in the dermis.

In addition to the established levels of heterogeneity within the dermal fibroblast compartment, both internal and external environmental influences have roles to play in shaping the fibroblast populations in skin. For instance, various characteristics of the skin differ in anatomically specialized regions: the papillary dermis is considerably thicker in certain regions of the body such as the palmoplantar regions (Cormack, 1987). Moreover, ageing has a profound impact on the skin, which becomes progressively thinner, losing dermal papillae of the epidermis and experiencing a reduction of the papillary dermis (Gilchrest, 1996). Interestingly, papillary dermal fibroblasts appear to be more affected by ageing than their reticular counterparts: with increasing age, the fibroblasts of the papillary dermis become more heterogeneous in terms of their size and display decreased proliferative rates in culture; papillary dermal fibroblasts also express increasing amounts of growth factors, including vascular endothelial growth factor (VEGF) with age, while reticular fibroblasts do not (Mine et al., 2008).

A final layer of complexity is added by the understanding that, following injury, the local need for replenishment of the dermal fibroblast compartment can be met either by proliferation of existing fibroblasts, from mesenchymal stem cell (MSCs) or from epithelial cells *via* a process of epithelial-mesenchymal transition (Strutz et al., 1995). Two mesenchymal cell types that deserve attention are MSCs and perivascular smooth muscle cells (pericytes) (Caplan, 2007; Mills et al., 2013; Paquet-Fifield et al., 2009). Both these mesenchymal populations express certain markers expressed by fibroblasts (that includes CD90, CD73, CD105, PDGFRα) (Ishii et al., 2005). Studies in mice and humans, suggest that activated fibroblasts or myofibroblasts can arise from resident and/or circulating mesenchymal stem cell (MSC) populations which originate from the bone marrow, adipose tissue or from pericytes associated with dermal microvasculature (Badiavas et al., 2003; Kataoka et al., 2003; Mills et al., 2013; Paquet-Fifield et al., 2009; Toma et al., 2001). Though these studies demonstrate the contribution of circulating MSCs in wound healing, recent lineage tracing and bone marrow transplantation studies in the mice do not indicate their contribution to dermis during development of skin (Barisic-Dujmovic et al., 2010; Driskell et al., 2013; Higashiyama et al., 2011).

In summary, the skin fibroblast compartment is comprised of several distinct sub-populations and perhaps also some intermediate states between these distinct groups. This heterogeneity results from differences in embryonic origin, anatomic site and state of differentiation, as well as micro-environmental influences and physiological processes such as ageing. Further, the differences in morphological, biochemical and functional profiles of various fibroblast populations impact keratinocyte growth and differentiation, and hence, homeostasis of the skin.

3. Insights from hair follicle associated fibroblasts

Hair follicle is a reservoir of stem cells that have the potential to regenerate hair and the epidermis. Investigations suggest the existence of stem cell populations within the epithelial outer root sheath (hair follicle epithelial stem cells) and within the dermal components of the hair follicle (FDP and DS) (Jahoda et al., 2003; McElwee et al., 2003; Taylor et al., 2000). The fibroblasts within the FDP and DS are believed to play an important role in induction, homeostasis and regeneration of epithelial structures including hair and epidermis (Gharzi et al., 2003; McElwee et al., 2003; Messenger et al., 1986). Analysis of cytokinetic dynamics within FDP and DS suggests bidirectional cell trafficking between the two compartments during normal hair cycle in mouse and human hair follicles (Jahoda, 2003; Tobin et al., 2003; Whiting, 2001). These studies also suggest the existence of progenitors within the DS that serve as a reservoir for the FDP fibroblasts.

The hair inductive properties of the hair follicle associated fibroblasts has been excellently demonstrated by studies using rat vibrissa or whisker follicles. Implantation of FDP into afollicular skin or beneath amputated vibrissa hair follicles induces new hair formation (Jahoda, 1992; Oliver, 1967, 1970). Similarly, cultured FDP and DS cells also possess the potential to reconstitute a new FDP *in vivo* (Horne and Jahoda, 1992; Jahoda et al., 1984; McElwee et al., 2003; Reynolds et al., 1999). In one of the seminal studies, implantation of DS from male human scalp into arm of female recipient resulted in growth of a new hair follicle with a FDP containing Y-chromosome (Reynolds et al., 1999). These observations suggest the hair inducing ability and probably immune privilege of FDP and DS fibroblasts.

Based on circumstantial evidence from dermal wound healing studies in animals and human surgical procedures, Jahoda and Reynolds (2001) provided two hypotheses. First, the DS fibroblasts could act as specialized progenitor populations that play a role in dermal wound healing. Second, involvement of DS fibroblasts in the wound healing process reduces the likelihood of scarring. To substantiate the hypotheses, Jahoda and Reynolds (2001) provided clinical evidences that human scalp skin heals faster compared to other parts of the body; bald scalp heals slower; ability to obtain multiple split-thickness grafts from the same donor site on the scalp skin; and relative lack of occurrence of hypertrophic scars and keloids on the scalp. Hence, the relative paucity of hair on most parts of the human skin could probably contribute to inferior wound healing and scar formation. Furthermore, they suggested that these DS fibroblasts are an easily accessible source of cells that

represent a residual embryonic-type fibroblast population within the mature skin; and incorporating these cells into full-thickness skin substitutes could improve wound healing and reduce scar formation (Jahoda and Reynolds, 2001). Recently, two studies have investigated the potential of using DS fibroblasts for reconstruction of skin substitutes. Cho et al. (2004) compared the ability of human DS fibroblasts and dermal fibroblasts to support epidermal morphogenesis *in vitro* using a collagen-based organotypic skin model. Histologically, the reconstructed skin using DS fibroblasts displayed thicker epidermis, stronger expression of filaggrin, keratin-1 and integrin-α6, a lower number of PCNA-positive cells, and weaker transferrin receptor staining compared to those with dermal fibroblasts. These observations suggest the superior ability of DS fibroblasts to support epidermal morphogenesis. However, a recent study comparing human FDP, DS and dermal fibroblasts cultured within collagen sponges and transplanted onto a full-thickness defect on the back skin of mice showed no significant difference in the wound healing abilities between the three fibroblast subtypes (Shin et al., 2011). However, the synthesis of new collagen was superior in the wounds grafted with FDP and dermal fibroblasts compared to DS fibroblasts suggesting a superior dermal architecture in the former. Further, the basement membrane formation based on expression of tenascin was superior in skin grafts with FDP fibroblasts compared to DS or dermal fibroblasts. Considering the easily accessibility and embryonic-like nature of hair follicle associated fibroblasts, further studies are needed to establish the probable benefits of these fibroblast subtypes proposed by Jahoda and Reynolds (Jahoda and Reynolds, 2001).

4. Insights from *in vitro* skin models

The clinical need to treat burns and chronic wounds, resulted in the development of the first human skin equivalents in the early 1980s (Bell et al., 1981, 1983). Following this pioneering efforts, there has been several publications on successful reconstruction of human skin equivalents *in vitro*. Briefly, fibroblasts are encapsulated within ECM components like collagen, fibrin or seeded onto de-cellularized dermis and cultured for a few days to a week. Then the keratinocytes are seeded on top of the dermal equivalents, cultured under submerged conditions for few days and then the culture is exposed to the air (commonly referred to as air–lift or air–liquid interface) resulting in epidermal differentiation, stratification and development of a cornified layer (Fig. 3). The various dermal substitutes used for reconstruction of full-thickness human skin equivalents include de-cellularized/de-epidermized dermis (El Ghalbzouri et al., 2002b; Lamb and Ambler, 2013; Lee and Cho, 2005), collagen type I (Moulin et al., 1999), collagen-gycosaminglycans (Varkey et al., 2011, 2014a,b), fibrin (Boehnke et al., 2007; Muffler et al., 2008; Stark et al., 2004b), fibroblast-derived matrix (Janson et al., 2013), and synthetic polymers (Boehnke et al., 2007). Based on these studies, various companies and start-ups have developed *in vitro* skin tissue equivalent models for pharmaceutical, cosmetic and chemical compound screening and testing. These commercially available skin tissue equivalents for *in vitro* testing are listed in Table 2.

In recent years, these full-thickness organotypic cultures have been increasingly employed in skin research. These models offer significant advantages over the traditional monolayer cocultures but also differ in important ways from natural skin tissue: organotypic cultures predominantly utilize mitotically-active early passage fibroblasts derived from human foreskins, abdomen, forearm or breast tissues which proliferate within the dermal matrix resulting in 3–4 fold increase over a 3 week culture period (Boehnke et al., 2007; Maas-Szabowski et al., 2000). As the majority of fibroblasts in normal skin are generally in a resting non-proliferative state, this represents a potentially significant difference and also introduces the additional variable of changing numbers of fibroblasts within the culture period, which impedes investigation of epithelial-mesenchymal interactions. Certain studies have explored the potential for combining the advantages of the organotypic approach with the added control of employing irradiated fibroblasts to populate the dermal matrix (Maas-Szabowski et al., 2000). The authors show that the morphology of the reconstructed epidermis is similar in organotypic co-cultures of keratinocytes with irradiated or non-irradiated dermal fibroblasts, and that problematic contraction of the collagen-based dermal matrix is less when irradiated fibroblasts are used. Hence, non-proliferative inactivated PMFs might be advantageous for use in organotypic cultures for several reasons: they more closely replicate the resting state of fibroblasts in native dermis, they reduce shrinkage of collagen gels, and their population size remains stable within the dermal matrix over the culture period (Maas-Szabowski et al., 2000). However, using PMFs for long-term cultures of over 3–4 weeks may not be suitable due to decrease in their numbers over the long culture period. As described above, native skin is composed of a ratio of MFs and PMFs, which is required for biochemical homeostasis of the dermis. Hence, it would be interesting to investigate the influence of specific ratios of MFs and PMFs in organotypic cultures on dermal and epidermal architecture.

Regardless of the source and mitotic state of fibroblasts used, their importance in driving optimal epidermal morphogenesis in engineered skin substitutes is clear. Collagen-based skin equivalents lacking fibroblasts exhibit an atrophic epithelium which is poorly organized and lack cornification (Boehnke et al., 2007; Contard et al., 1993; El Ghalbzouri et al., 2002b, 2004). Similarly, in fibrin-based skin equivalents, the absence of fibroblasts is associated with an atrophic epithelium possessing just 3–4 layers of keratinocytes, lack of a defined stratum granulosum, and display the parakeratinization (presence of nuclear remnants in the cornified layer) instead of orthokeratinization. Reinstating a fibroblast compartment induces the development of 5–8 epidermal layers with well-defined stratum granulosum and a uniform orthokeratinized layer (Fig. 4). Further, studies also reveal the importance of fibroblast-keratinocyte cross-talk for the generation of a well-organized basement membrane, which was conventionally thought to be a purely keratinocyte-driven process (for excellent reviews on basement membrane organization and epidermal morphogenesis see, Breitkreutz et al., 2013; McMillan et al., 2003; Miner, 2008; Miner and Yurchenco, 2004).

Studies on organotypic cultures on type I collagen and de-epidermized dermis have demonstrated the role of keratinocyte-fibroblast interactions that influence the formation of basement membrane and which in turn can influence the keratinocyte phenotype (El Ghalbzouri et al., 2004). While keratinocytes produce the majority of the components of the basement membrane, expression of bullous pemphigoid (BP) antigens BP230 and BP180, laminins and type IV collagen are weak and delayed in the absence of fibroblasts (El Ghalbzouri et al., 2005; Lee and Cho, 2005; Okamoto and Kitano, 1993; Schafer et al., 1991). Moreover, we now know that certain components of the basement membrane like laminin-10, nidogens/entactin are produced primarily by dermal fibroblasts (Contard et al., 1993; Fleischmajer et al., 1995; Mokkapati et al., 2008; Nischt et al., 2007), while components like laminin-5 and collagen VII are primarily produced by keratinocytes (Marinkovich et al., 1993), and other components are produced by both cell types through mutual interactions. However, the primary role of fibroblasts appears to be their instructive effects on keratinocytes, promoting both the production of basement membrane components and appropriate organization of the basement membrane zone (Lee and Cho, 2005). Other studies have similarly emphasized the importance of fibroblast-keratinocyte communication in

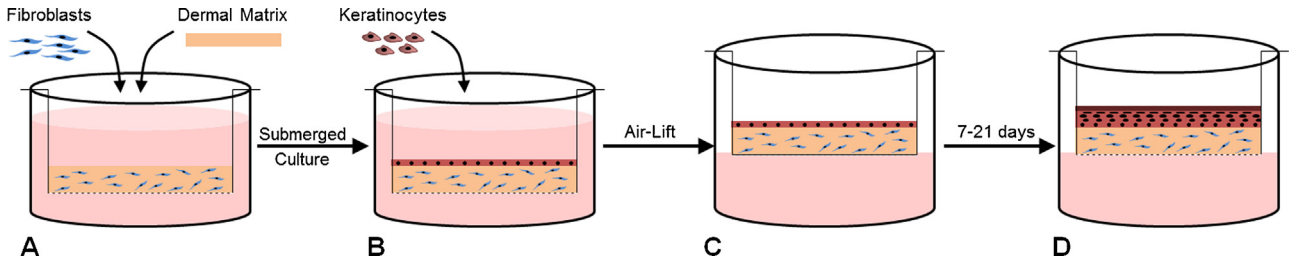

Fig. 3. Schematic representation of culture of organotypic skin model. Dermal matrices with or without fibroblasts are cultured under submerged conditions within a culture insert (A). After a few days, keratinocytes are seeded on top of the dermal matrices (B). After a few days of submerged culture of keratinocytes, the culture is exposed to air allowing the media to perfuse only through the dermal side (C). This process of culturing the keratinocytes at an air–liquid interface results in their differentiation, formation of a stratified and cornified epidermis (D).

Table 2
Commercially available organotypic skin equivalents for in vitro testing and related applications.

	Brand name	Manufacturer/service provider	Cells	Scaffold
Epidermis models	epiCS (EST-1000)	CellSystems (Germany)	Human keratinocytes	Polycarbonate membrane
	Epiderm	MatTek Corp. (USA)	Human keratinocytes (neonatal/adult)	Collagen-coated polycarbonate membrane
	Leiden epidermal skin model (LEM)	Biomimiq (Netherlands)	Human keratinocytes (neonatal/adult)	Inert acellular filter
	SkinEthic RHE	EpiSkin (France)	Human keratinocytes (neonatal/adult)	Polycarbonate membrane
	epiCS-M	CellSystems (Germany)	Human keratinocytes with human melanocytes from donors of three different phototypes: Caucausian, Afro-American and Asian-Caucasian	Polycarbonate membrane
	MelanoDerm	MatTek Corp. (USA)	Human keratinocytes and human melanocytes from donors of three different phototypes: African, Caucausian and Asian	Inert cell culture insert membrane
	SkinEthic RHPE	EpiSkin (France)	Human keratinocytes and human melanocytes	Inert cell culture insert membrane
Full thickness skin models	3D HSE	MD Biosciences (Switzerland) and Fraunhofer IGB (Germany)	Human keratinocytes and fibroblasts	Proprietary: Biomatrix consisting of tissue-typical matrix proteins
	AST-2000 (discontinued)	CellSystems (Germany)	Human keratinocytes and fibroblasts	Collagen
	Epiderm-FT	MatTek Corp. (USA)	Human keratinocytes and human fibroblasts (neonatal/adult)	Collagen
	EpiSkin	EpiSkin (France)	Human keratinocytes (neonatal/adult)	Collagen
	Fibroblast-derived matrix model (FDM)	Biomimiq (Netherlands)	Human keratinocytes and fibroblasts (neonatal/adult)	Human fibroblast-seeded dermal matrix
	Full thickness model (FTM)	Biomimiq (Netherlands)	Human keratinocytes and fibroblasts (neonatal/adult)	Human fibroblast-seeded collagen matrix
	Phenion FT	Henkel AG & Co. (Germany)	Human keratinocytes and fibroblasts (neonatal foreskin)	Bovine, cross-linked lyophilized collagen
	StrataTest	Stratatech (USA)	Immortalized human keratinocyte progenitor cell line (NIKS) and dermal fibroblasts	Collagen I
	Vascularized 3D HSE	MD Biosciences (Switzerland) and Fraunhofer IGB (Germany)	Human keratinocytes, fibroblasts and microvascular endothelial cells	3D HSE vascularized with BioVasc technology (decellularized jejunum seeded with microvascular endothelial cells)
Iseased skin models	Melanoma tissue model (MLNM-FT-A375)	MatTek Corp. (USA)	Human malignant melanoma (A375) cells combined with EpiDerm-FT tissues	Collagen
	Psoriasis tissue model (SOR-300-FT)	MatTek Corp. (USA)	Normal human keratinocytes with psoriatic fibroblasts within EpiDerm-FT tissues	Collagen

basement membrane formation, rather than a predominant role of just one cell type (Fleischmajer et al., 1998; Smola et al., 1998). For instance, fibroblasts express TGFβ2 upon induction by keratinocytes, and this TGFβ2 in turn regulates the production of collagen type VII and laminins by keratinocytes locally in a paracrine manner (König and Bruckner-Tuderman, 1991, 1992, 1994; Smola et al., 1994, 1998). Further, in organotypic cultures, collagen type IV and VII, laminin-5, laminin-10/11, nidogens, and uncein are expressed only in the presence of fibroblasts or fibroblast-expressed soluble factors like EGF, KGF and GM-CSF (El Ghalbzouri et al., 2005; Smola et al., 1998). Studies on organotypic mono-/cocultures of human keratinocytes and fibroblasts have demonstrated that collagen VII (an integral part of anchoring fibrils) is primarily produced by keratinocytes; and collagen VII expression is highly upregulated in the presence of fibroblasts and TGFβ2 (König and Bruckner-Tuderman, 1991, 1992, 1994; Smola et al., 1998; Stark et al., 2004b). Similarly, studies on mice and organotypic cultures have demonstrated the role of perlecan, laminins, and nidogens-1 and -2 in the assembly of basement membrane components (Bader et al., 2005; Mokkapati et al., 2008, 2011; Nischt et al., 2007; Sher et al., 2006). Sher et al. (2006) investigated the role of perlecan in the formation of human epidermis using organotypic cultures.

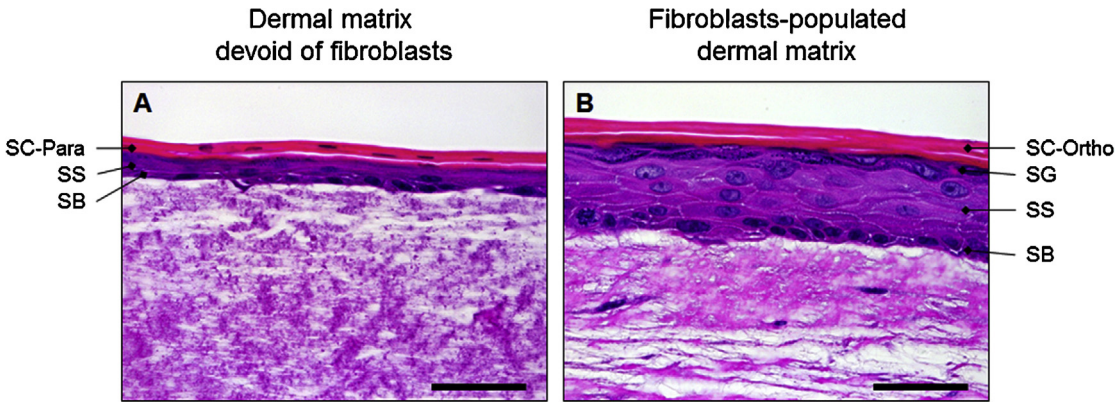

Fig. 4. Organotypic culture of N/TERT-1 keratinocytes over fibrin-based dermal matrices without fibroblasts under serum-free, defined conditions result in an epithelium that is atrophic, lacks a defined stratum granulosum, and exhibits para-keratinization (SC-Para) (A). On other hand culture over fibroblast populated fibrin-based dermal matrix results in a well-organized epidermis with well-defined stratum granulosum (SG) and a uniform layer of orthokeratin (SC-Ortho) (B). SB—stratum basale; SS—stratum spinosum. Scale bar: 50 μm.

Organotypic cultures with fibroblasts lacking perlecan displayed no effect on basement membrane organization and epidermal morphology. However, organotypic cultures with perlecan-deficient keratinocytes displayed atrophic and poorly organized epidermis due to enhanced apoptosis. Similarly, nidogen plays a part in assembly of basement membrane components, through its role as a linker joining laminin and collagen IV. Lack of both nidogen -1 and -2 in organotypic cultures of human keratinocytes and murine fibroblasts (with varying nidogen deficiency) prevented the deposition and ultrastructural assembly of basement membrane, while the expression or supplementation of either isoform restored the basement membrane structure suggesting the role of fibroblasts in nidogen expression and in turn basement membrane organization (Mokkapati et al., 2008, 2011; Nischt et al., 2007). Additionally, interaction between laminin and nidogen may have an influence on the epidermal stem cell phenotype (Paquet-Fifield et al., 2009; Pouliot et al., 2002; Pujuguet et al., 2000). Hence, fibroblasts play a direct and a paracrine role in synthesis, assembly and organization of the basement membrane; and organotypic cultures provide a valuable platform to study basement membrane organization *in vitro*.

Given the multiple and varied roles of fibroblasts in the skin, it is perhaps not surprising that the precise conditions of their inclusion in *in vitro* co-culture models has a profound effect on the resulting epidermis. Initial investigations in organotypic cultures indicate that increasing concentrations of fibroblasts conferred improved epidermal architecture (El Ghalbzouri et al., 2002a). Low fibroblast concentrations (2×10^4 fibroblasts/ml) are associated with expression of involucrin in the suprabasal layer, and the presence of the keratinocyte activation markers K6, K16 and K17 in a pattern normally seen in regenerating epidermis; while at higher concentrations (8×10^4 fibroblasts/ml) involucrin expression is restricted to the stratum granulosum and K6, K16 and K17 are not expressed, similar to native human skin (El Ghalbzouri et al., 2002a; Stark et al., 1999). Attempts to optimize such variables as timing of keratinocyte addition to the co-culture, fibroblast preconditioning and fibroblast densities suggest that overnight preconditioning of fibroblast-populated collagen matrices (before keratinocyte seeding) are associated with lower collagen matrix contraction with increasing fibroblast densities; while 1-week preconditioning induces marked matrix contraction irrespective of fibroblast concentration (El Ghalbzouri et al., 2002a). As explained already, organotypic culture of full-thickness skin equivalents principally involves the following steps: seeding and culture of fibroblasts onto/within dermal matrices followed by keratinocyte seeding and culture at air–liquid interface. The initial phase of culturing fibroblasts encapsulated within/seeded on dermal matrices prior to the addition of keratinocytes is referred to as fibroblast pre-conditioning. Similarly, we observed contraction of collagen-based dermal matrix with and without the presence of fibroblasts, while contraction of the fibrin-based dermal matrix was minimal irrespective of the presence or absence of fibroblasts (Fig. 5). However, studies using fibrin-based dermal matrices suggest that pre-conditioning for 2 days results in superior epidermal morphogenesis and basement membrane development compared to 7 and 14 days of fibroblast pre-conditioning (Boehnke et al., 2007). Experiments with scaffold-reinforced fibrin-based dermal matrices and extended culture periods of up to 12 weeks have previously been reported (Boehnke et al., 2007; Muffler et al., 2008), highlighting the inherent properties of different dermal matrices and the potential enhancements that may be made to culture models under the right conditions.

Interestingly, the role of fibroblast heterogeneity in epidermal morphogenesis of organotypic skin equivalents is increasingly recognized. Papillary dermal fibroblasts appear to induce basement membrane formation more rapidly in 3D cocultures than the site-matched reticular dermal population (Sorrell et al., 2004). Hence, it is possible that the fibroblast subpopulations closest to the epidermis either produce more basement membrane components or higher amounts of the paracrine factors that drive keratinocyte to establish and organize the basement membrane. In addition, to the role of fibroblast heterogeneity in basement membrane organization, studies highlight their role in epidermal organization. It is well established that balanced production of various paracrine factors like KGF-1, GM-CSF, and IL-1α is required for appropriate keratinocyte differentiation and epidermal morphogenesis (Maas-Szabowski et al., 2001; Sorrell et al., 2004). Cultured papillary dermal fibroblasts secrete higher levels of GM-CSF to KGF-1 compared to site-matched reticular dermal subpopulations (Sorrell et al., 2004). Given that fibroblast subpopulations can express different levels of these bioactive factors, it is imperative that the most appropriate fibroblast subpopulation is identified for use in specific skin tissue engineering applications.

Differences in the ECM and fibroblasts of papillary and reticular dermis may play a role in engineering skin equivalents. The work by Lee and Cho (2005) has shown the importance of these layers of dermis on epithelization in culture (Lee and Cho, 2005). Organotypic culture of keratinocytes on reticular layer of decellularized dermis gives rise to multi-layered epidermis without cornification, the basal keratinocytes are flat and arranged parallel to dermo-epidermal junction, and components of basement membrane are poorly expressed and organized. On

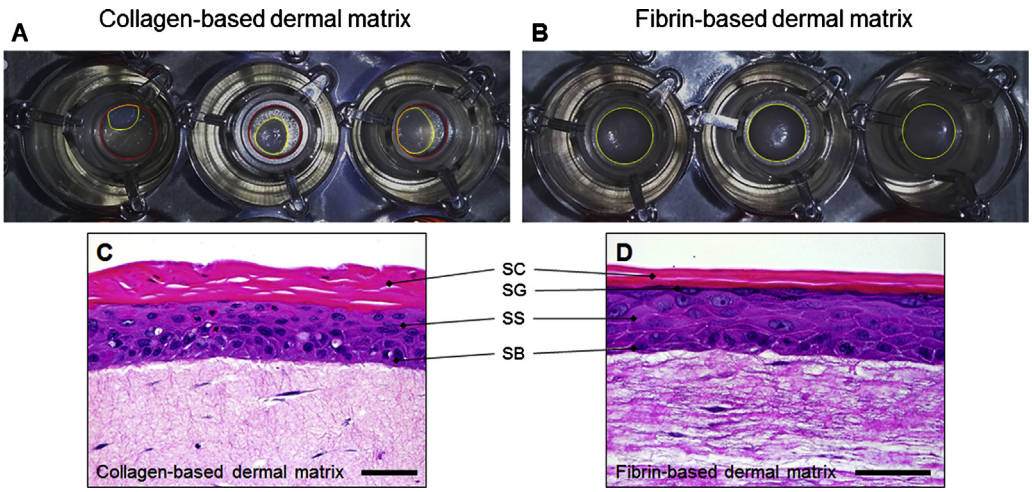

Fig. 5. Organotypic cultures of N/TERT-1 keratinocytes over fibroblast populated collagen-based dermal matrix under serum-free defined conditions results in variable contraction of the culture (A) as could be visualized by the difference in area enclosed in red (original area occupied by the gel before contraction) and in yellow (area occupied by the gel after contraction). On the other hand, organotypic cultures on fibroblast populated fibrin-based dermal matrix show almost no contraction (B). The lower panel shows the histological picture of the organotypic skin formed after 3 weeks of culture on collagen (C) and fibrin (D) based dermal matrices. SB—stratum basale; SS—stratum spinosum; SG—stratum granulosum, SC—stratum cormeum. Scale bar: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the other hand, culture of keratinocytes on the papillary layer of decellularized dermis show multi-layered epithelium with thick cornified layer similar to that observed *in vivo*, the basal keratinocytes are polarized with their long axes oriented perpendicular to the dermo-epidermal junction; and characterized by expression of well-organized deposition of basement membrane components (collagen-IV, collagen-VII, integrin-β4, laminin, and tonofibril-hemidesmosome complexes). Similarly, Varkey et al. (2011, 2014a,b) in a series of publications have demonstrated the superiority of papillary (superficial) fibroblasts compared to reticular (deep) dermal fibroblasts for construction of tissue engineered skin equivalents. Collagen-glycosaminoglycan matrices cultured with reticular dermal fibroblasts demonstrate higher contraction and stiffness of the matrix compared to those with papillary fibroblasts; in addition the matrices with reticular fibroblasts show significantly higher α-SMA staining (probable differentiation to myofibroblast-like phenotype), higher production of pro-fibrotic factors TGFβ1 and osteopontin (Varkey et al., 2011, 2014a). Further, the culture of keratinocytes with superficial dermal fibroblasts result in superior epidermal morphogenesis in terms of higher expression levels of proteins related to epidermis and basement membrane, are less fibrotic, and has superior epidermal barrier function compared to those cultured with deep dermal fibroblasts (Varkey et al., 2014a,b). Similarly, a recent study demonstrated the differential regulation of epidermal proliferation and terminal differentiation by papillary and reticular fibroblasts in fibroblast-derived matrix organotypic cultures (Janson et al., 2013). The skin equivalents with papillary fibroblasts demonstrate superior epidermal morphogenesis as revealed by defined basal layer with polarized basal keratinocytes, and defined stratum granulosum with stronger expression of filaggrin and loricrin, and a thick corneal layer. Further, organotypic cultures with papillary fibroblasts demonstrated increased synthesis of MMP-1 and VEGF, while those with reticular fibroblasts showed higher levels of MMP-3 and KGF synthesis (Pageon et al., 2012). Together these studies suggest that primary fibroblast isolation from the dermis should take into account the differences in the papillary and reticular dermal fibroblasts, and that appropriate populations of fibroblasts should be isolated and used for engineering skin substitutes rather than a heterogeneous mixture of dermal fibroblasts.

As the role of inherent differences in the papillary and reticular dermal fibroblast phenotypes is increasingly recognized, another factor that influences the difference between these two phenotypes is also beginning to be understood. Interestingly, studies have observed differences in the papillary and reticular dermal fibroblasts with physiological ageing and its impact on epidermal morphogenesis. Site-matched studies on skin biopsies obtained from young and old donors show thinner epidermis with shorter rete ridges, flatter dermo-epidermal junction and more disorganized collagen fibers in the dermis in the older individuals (Mine et al., 2008). Further, *in vitro* studies show that site-matched papillary dermal fibroblasts show that these dermal fibroblasts from older individuals are more heterogeneous in their size profile and have a slower proliferation rate. Accordingly, aging papillary fibroblasts (*in vitro*) exhibited upregulation in the secretion profiles of KGF, VEGF, MMPs (-1, -2 and -3) and tissue inhibitor of metalloproteinases (TIMP-1 and TIMP-2), while there is no difference among the reticular fibroblasts with age (Mine et al., 2008). Organotypic cultures of these dermal fibroblasts also exhibit differences in their ability to contract. Papillary dermal fibroblasts from older donors contract collagen gels more than those from younger donors, while again there is no difference among the reticular dermal fibroblasts with age. Finally, organotypic cocultures of keratinocytes and fibroblasts, show thicker epidermis with well-defined stratum corneum and orthokeratinization in cultures with papillary dermal fibroblasts from younger donors; while those with papillary dermal fibroblasts from older donors had thinner epidermis and stratum corneum (similar to those cultures with young or old reticular dermal fibroblasts). These differences were also evident upon transplantation into nude mice (Mine et al., 2008). Hence, the aging of the papillary dermal fibroblasts might account for the epidermal atrophy and loss of rete ridges with age. Similarly, another study has highlighted the effect of aging induced by long-term culture of the fibroblasts on the epidermal morphogenesis. Equivalents with early-passage papillary fibroblasts demonstrated well organized epidermis, while those with late-passage papillary fibroblasts resembled those with reticular fibroblasts (Janson et al., 2013). Interestingly, the skin equivalents with early-passage fibroblasts lacked the expression of keratin-16 (a marker associated with activated keratinocyte phenotype) indicating the superiority of these skin equivalents. Additionally, organotypic cultures with

late passage reticular fibroblasts were accompanied by atrophic epithelium. These results suggest the probability of differentiation of papillary fibroblasts to reticular fibroblast-like phenotype upon long-term culture accompanied by poorer ability to support epidermal morphogenesis and resemblance to a more 'aged' phenotype. Taken together, these studies indicate that future attempts at skin tissue engineering should consider the phenotype of dermal fibroblasts, age of the donor, duration of *in vitro* culture, the expression of reticular fibroblast/myofibroblast-related markers like α-SMA, and/or expression of paracrine, pro-fibrotic, and anti-fibrotic factors.

In addition to the fibroblasts from dermis and FDP, fibroblasts derived from other sources such as the pericytes surrounding the dermal microvasculature deserve consideration. These perivascular cells possess mesenchymal stem cell-like properties with propensity to differentiate to osteogenic, chondrogenic and adipogenic lineages in addition to their ability to be activated to fibroblast-like cells. A recent study investigated the effect of pericytes incorporated within organotypic cocultures with human keratinocytes (Paquet-Fifield et al., 2009). Coculture with pericytes resulted in enhanced deposition of laminin-511/521 in the dermal-epidermal junction. More importantly, in contrast to dermal fibroblasts, pericytes were demonstrated to rekindle the tissue regenerative capacity of human keratinocytes committed to differentiation (Integrin-$\alpha6^{dim}$ epidermal cells) probably through their secreted factors. This finding indicates superior support offered by the pericytes in organotypic cocultures and probably in wound healing. However, studies in human wounds suggest that pericytes unlike other MSCs are more likely to promote fibrosis than to accelerate wound healing (Sundberg et al., 1993). Further studies on pericyte-derived fibroblasts are necessary to understand their role in skin homeostasis.

Recently, attention has been focused on understanding the neuro-endocrine properties of human skin that includes the expression and function of specific receptors and synthesis of hormones, neurotransmitters and steroids. In this section of the review, the role of various neuro-endocrine properties and non-neuronal receptor systems are briefly discussed. The hormonal receptor system includes the expression of parathyroid hormone/parathyroid hormone-related peptide, thyroid stimulating hormone, corticotropin-releasing hormone, melanocortin and melatonin receptors. Though investigations suggest the existence of these receptors in epidermal and/or dermal compartments, they have been primarily focused on skin keratinocytes and information on their role and expression among the heterogeneous dermal fibroblasts is limited. Studies suggest the expression of neurohormone receptors and neurotransmitter systems in non-neuronal skin cells which are involved in skin homeostasis and inflammation (for excellent reviews refer to Grando et al., 2006; Kurzen et al., 2007; Raap and Kapp, 2010; Roosterman et al., 2006; Truzzi et al., 2011). These include acetylcholine (nicotinic and muscarinergic), adrenergic, histamine, opioid, cannabinoid, kinin, prostanoid, vasoactive intestinal peptide, somatostatin and neuropeptide-Y receptors. Nicotinergic and mucarinergic acetyl choline receptors (nAChR and mAChR) are reported to control epidermal homeostasis and terminal differentiation *in vitro* and in skin of mice (Arredondo et al., 2002, 2003b; Nguyen et al., 2004). Organotypic cocultures of human keratinocytes and fibroblasts treated with nicotine display hyperplastic epidermis; and blockade of nAChR lead to delay in epidermal proliferation and differentiation evidenced by reduced epidermal thickness and terminal differentiation markers (Hana et al., 2007; Kurzen et al., 2006). Though these investigations have primarily focussed on epidermis, exposure of dermal fibroblasts to nicotine results in increase in cell cycle and apoptosis related regulators; and upregulation of MMP-1 and collagen type-I suggesting an alteration in ECM turnover which in turn could affect the epidermal morphogenesis (Arredondo et al., 2003a). We and others have established the role of opioid receptors in skin homeostasis and differentiation (discussed in detail under the section on keloids) (for reviews see, Bigliardi and Bigliardi-Qi, 2014; Bigliardi et al., 2009). Similarly, studies have observed that adrenergic receptors play a role in the pathophysiology of various skin diseases such as psoriasis, atopic dermatitis, vitiligo and scleroderma through their effect on keratinocyte migration and wound reepithelization (Grando et al., 2006; Sivamani et al., 2007).

Another group of receptors expressed in the skin are the nuclear receptors that include glucocorticoid, androgen, progesterone, thyroid hormone, estrogen, vitamin D, peroxisome proliferative-related receptors (PPAR), retinoid acid (RAR) and retinoid X (RXR) receptors. The importance of these receptor systems in epidermal morphogenesis have been recognized by earlier studies including hydrocortisone, triiodothyronine, vitamin D, retinoic acid, and progesterone as media supplements for *in vitro* organotypic skin cultures (Asselineau et al., 1989; Crowe et al., 1991; Gangatirkar et al., 2007; Isseroff et al., 1989; Kaplan et al., 1988; Sorensen et al., 1997). Glucocorticoids are well known to induce epidermal atrophy and hair growth, presumably due to their effects on dermal fibroblasts (Gysler et al., 1997; Stratakis et al., 1998). Studies on human skin and monolayer keratinocyte cultures show that glucocorticoids regulate keratinocyte differentiation by suppressing the expression of basal cell specific keratins (K5 and K14), and keratins associated with activated phenotype (K6, K16 and K17) (Radoja et al., 2000). Androgen and estrogen receptors are reported to be strongly expressed in both keratinocytes (epidermal and follicular keratinocytes) and fibroblasts (dermal and follicular). Though investigations suggest that androgens stimulate the proliferation of FDP fibroblasts, excessive amounts of androgens are shown to induce apoptosis of cultured human FDP fibroblasts through the bcl-2 pathway and hence, play a role in androgenetic alopecia (Randall et al., 1992; Winiarska et al., 2006). Estrogens on the other hand, are well known to improve the quality of human skin by their effects on collagen content (increased total collagen and ratio of type III:I collagen), stratum corneum (increased water-holding capacity), hair growth, enhanced vascularization and increased skin thickness (Brincat, 2000; Thornton, 2002; Verdier-Sevrain et al., 2006). Considering the effects of androgens and estrogens on epidermal homeostasis, it would be interesting to investigate their effects in organotypic cultures. Similarly, *in vitro* studies on monolayer and organotypic skin cultures have shown the effects of retinoic acid on keratinocyte differentiation and proliferation in normal and diseased human skin (Asselineau et al., 1989; Asselineau and Darmon, 1995; Choi and Fuchs, 1990; Fuchs and Green, 1981; Jean et al., 2011; Kautsky et al., 1995). Further, studies have demonstrated that retinoic acid modulates the epithelial differentiation through its effects on expression of keratins K19, K1/K10 and K4/K13 (Crowe et al., 1991; Kopan et al., 1987; Schon and Rheinwald, 1996). Studies on organotypic cultures and humans, have demonstrated that retinoids promote proliferation in normal human skin, while they have an anti-proliferative effect with normalization of the differentiation process in psoriatic skin (Asselineau and Darmon, 1995; Giltaire et al., 2009; Gottlieb et al., 1996; Heise et al., 2006; Jean et al., 2011). Similarly, *in vitro* studies have shown the anti-proliferative and pro-differentiating effects of vitamin D3 and triiodothyronine (Isseroff et al., 1989; Sorensen et al., 1997).

In summary, over the recent years and with the advent of organotypic models our knowledge of skin biology and of the roles of fibroblasts/fibroblast-keratinocyte cross-talk has grown significantly. However, even the best *in vitro* models of native skin cannot be expected to recapitulate all aspects of the complexity of the *in vivo* situation, for instance, the impacts of barrier property, innervation, a continuous blood supply, presence of skin appendages,

and interactions with immune cells. This is particularly relevant for the development of skin substitutes for clinical use, as they will invariably be required in cases where the normal architecture of the skin has been destroyed by wounding and heavy inflammation. Understanding the wound healing process is a pre-requisite to the design of biological skin replacements that are able to reinstate normal architecture and function to the affected region. For this, we turn our attention to studies of healing, and the roles played by various fibroblast subpopulations.

5. Insights from wound healing studies

Skin healing requires complex interactions between keratinocytes, fibroblasts, ECM, and stem cell populations within the basal epidermis, appendages and underlying mesenchyme. In adults, the healing of large and/or full-thickness wounds is invariably associated with the formation of scar tissue, and numerous studies indicate a role for fibroblasts in this process, which will be discussed below. Also of particular interest are the specialized situations where scarless wound healing occurs, such as in first and second trimester fetal skin (Lorenz et al., 1992, 1995), and the oral mucosa, where fibroblasts are pivotal in directing complete regeneration of the affected tissue. Thus understanding the mechanisms underlying the two ends of the wound healing spectrum will provide valuable insights into optimal engineering of replacement skin tissues favoring maximal skin regeneration with minimal scarring.

5.1. Roles of fetal fibroblasts in scarless healing

The early-gestation fetus has a unique ability to completely heal skin wounds without forming a scar (Lorenz et al., 1993; Rowlatt, 1979). Hence, there has been much interest in understanding the factors and mechanisms underlying the scarless healing pattern observed with the aim of incorporating fetal factors in refined skin tissue engineering protocols. Initially it was proposed that aspects of the uterine environment and/or components of the amniotic fluid, which is rich in growth factors and ECM molecules, were responsible for the scarless phenotype (Longaker et al., 1989, 1990a, 1991; Mast et al., 1993; Whitby and Ferguson, 1991b). However, neither the application of amniotic fluid nor the perfusion of fetal blood prevented scarring in wounded adult skin grafts (Longaker et al., 1994). Additionally, scarless wound healing is restricted to the first 24 weeks of human gestation, while after this time wounds heal with fibrosis and scarring despite a relatively similar intrauterine environment (Longaker et al., 1990b). The high regenerative capability of early to mid-gestational fetal skin has been demonstrated across many mammalian species including mice, rats, sheep, monkeys and humans (Adzick and Longaker, 1991, 1992; Ihara et al., 1990; Lorenz et al., 1992, 1993), indicative of an evolutionarily conserved mechanism. Furthermore, as gestation proceeds the ability to heal comparable wounds without scarring is progressively lost; in fetuses of non-human primates a sequential loss of ability to regenerate hair follicles and other appendages occurs in mid-gestation, followed by disorganized deposition of collagen following wounding in late-gestation (Lorenz et al., 1993). The concept that scarless healing is an inherent property of early-gestation fetal skin is further supported by the finding that subcutaneous transplantation of full-thickness human fetal skin grafts into adult athymic mice, followed by wounding results in scarless repair; while transplantation onto cutaneous fascial bed is associated with scarring (Lorenz et al., 1992). Detailed characterization of the implanted fetal skin indicate that it is the fetal fibroblast within the graft that is responsible for determining the scarless *versus* scarring phenotype (Lorenz et al., 1995), and thus the focus of research shifted to investigating the cellular and molecular mechanisms of fetal wound healing and how they differ from those in the adult.

In fact, fetal and adult fibroblasts are distinct in several important respects; fibroblasts from fetuses differ in their ability to synthesize and degrade ECM, and have higher proliferative and migratory activity that decreases with increasing age (Schor et al., 1985; Shiraha et al., 2000). The increased migration of murine fetal fibroblasts may relate to the earlier expression of fibronectin and tenascin, which promote rapid re-epithelialization following wounding (Carter et al., 2009; Whitby et al., 1991). Additionally, while wound healing in the adult is associated with initial proliferation of fibroblasts followed by synthesis of ECM, in the fetal wound, wound fibroblasts proliferate and deposit ECM simultaneously (Larson et al., 2010). The differences in healing dynamics and the reduced migratory ability of adult dermal fibroblasts and delay in deposition of collagen in adult wounds is believed to be an important factor in delayed wound healing and scar formation. As well as synthesizing ECM relatively earlier in the wound healing process, the composition of the matrix deposited by fetal fibroblasts is different; fetal fibroblasts (mice and rats) synthesize higher amounts of total collagen and a higher proportion of collagen type-III relative to type-I than do adult dermal fibroblasts (Carter et al., 2009; Hallock et al., 1988, 1993; Merkel et al., 1988). Second, 30–60% of the collagen in the dermis of fetal skin is collagen type-III, while in the adult dermis collagen type-III accounts for only 10–20% of the total collagen (Bullard et al., 2003). This difference in ratio between collagen types is potentially significant, as collagen type-III fibers are much shorter and finer than those of collagen type-I. Thus, the particular collagen composition of fetal skin is associated with deposition of collagen fibers in a fine reticular pattern with less cross-linking, as opposed to the thick, disorganized collagen fibers with extensive cross-linking that are synthesized by adult dermal fibroblasts (Longaker et al., 1990b; Whitby and Ferguson, 1991a). Scarless repair is associated with such reticular deposition of collagen types-I and -III leading to regeneration of dermal tissue that is indistinguishable from adjacent non-wounded dermis, while scar formation is characterized by the deposition of disorganized collagen fibers and the absence of epidermal appendages (Lorenz et al., 1995). These differences in cellular and extracellular factors influencing fetal and adult wound healing are highlighted in Table 3.

In addition to differences in collagen and adhesion molecule expression relative to adult wounds, fetal dermis and wounds are also rich in glycosaminoglycans, especially hyaluronic acid (HA). HA is a large hydroscopic molecule composed of alternating units of glucuronic acid and *N*-acetylglucosamine and has the ability to expand 1000 to 10,000 times its own volume by imbibing water which facilitates hydration of the ECM (Bullard et al., 2003): a matrix rich in HA is loose and permissive for cell adhesion, proliferation and migration. HA also acts as a growth factor 'sink' by binding various growth factors and cytokines, thereby modulating the temporal and spatial presentation of these factors to fibroblasts. Relative to their adult counterparts, fetal fibroblasts (rabbit/human) produce more HA, have 2–4 fold higher expression of HA receptors and produce greater amounts of HA synthases (Alaish et al., 1994; Kennedy et al., 2000). *In vitro*, fetal rabbit fibroblasts cultured in the presence of HA proliferate less and produce higher levels of collagenous and non-collagenous proteins (Mast et al., 1993), which may be especially relevant considering that *in vivo*, HA remains in the fetal lamb wound bed for longer periods than in adult wounds (Longaker et al., 1990a, 1991). HA may also be involved in the progressive loss of scarless healing ability with increasing gestational age; in fetal lamb wound fluid, the levels of HA and HA-stimulating activity reduce significantly during the transition period from fetal-type to adult-type wound healing (Estes et al., 1993). Additionally, the total HA content of fetal skin

Table 3
Comparison of factors influencing fetal and adult wound healing.

	Fetal wound healing	Adult wound healing
Extracellular matrix		
Collagen	Fine, reticular or basket-weave pattern; lower cross-linking	Thick; dense parallel bundles or disorganized; higher cross-linking
Collagen type I: type III (ratio)	Low	High
Collagen deposition (rate)	Immediate	Delayed
Hyaluronan and GAGs	Higher and persistent expression of HA and HA-receptor (CD44); higher molecular weight HA; high HASA; more nonsulfated GAGs	Lower and transient expression of HA and HA-receptor (CD44); lower molecular weight HA; low HASA; more sulfated GAGs
Tenascin-C	More; early deposition	Low; late deposition
Decorin	Low	High
Fibromodulin	High	Low
Fibronectin	More; early deposition	Low; late deposition
Cellular factors		
Epithelization	Rapid	Slow
Fibroblast	Faster migration	Slower migration
Myofibroblasts	Low and transient appearance during early wound phase	High and persistent (especially larger wounds)
Stem cells	High	Low
Inflammation		
Response	Minimal	Higher levels (leucocytes, macrophasges, mast cells)
Cytokines and chemokines	Lower levels of pro-inflammatory (IL6, IL8); Higher levels of anti-inflammatory (IL10); low levels of COX-2 and PGE2	Higher levels of pro-inflammatory (IL6, IL8); Lower levels of anti-inflammatory (IL10)
Growth factors		
TGF-β1	Low levels; transient	High levels; prolonged presence
TGF-β3	Higher levels; quick and prolonged expression	Delayed expression

COX2—cycloygenase-2; GAGs—glycosaminoglycans; HA—hyaluronic acid; HASA—hyaluronic acid stimulating activity; IL—interleukins; PGE2—prostaglandin-E2; TGF-β—transforming growth factor-β.

reduces with increasing fetal age, and this process is temporally linked with the appearance of other glycosaminoglycans, especially decorin and heparin sulphate (Estes et al., 1993). These temporal changes in the distribution of HA and other glycosaminoglycans reflects the change from a fetal to an adult-like phenotype of the ECM that plays an important role in scarless repair of fetal skin wounds mediated by fetal fibroblasts across a range of mammalian species.

More recently, the potential to use fetal fibroblasts in engineered skin substitutes has begun to be explored, and has shown some promise. Dermal matrices self-assembled by early human fetal fibroblasts *in vitro* resemble a mid-gestational fetal dermis in terms of various morphological and biochemical parameters including amount of collagen and HA (Pouyani et al., 2012). Furthermore, gene expression array analysis of fetal and neonatal dermal matrices, shows lower expression of genes associated with fibrosis and higher expression of genes associated with anti-fibrosis in fetal fibroblast compared to neonatal fibroblast-derived dermal matrix (Pouyani et al., 2012). Given the abundance of HA within the fetal dermis and fetal wounds, several studies have explored the use of HA-rich matrices in organotypic skin models: using esterified HA fibers and fibrin gels with fibroblasts as a dermal equivalent, Boukamp and colleagues demonstrated superior dermal and epidermal architecture and long-term stability of the full-thickness organotypic skin cultured for over 12 weeks relative to conventional collagen-based dermal equivalents (Boehnke et al., 2007; Muffler et al., 2008; Stark et al., 2004b, 2006). The fibroblasts within the above HA-fibrin based model also secrete a provisional matrix consisting of fibronectin and tenascin that progressively generated organized collagen types-I and -III during the culture period, resembling mature dermis.

Hence, a number of unique factors within the fetal microenvironment, in particular fetal fibroblasts and the surrounding ECM seem to contribute to scarless healing. Better understanding of the mechanisms that underlie the scarless fetal wound healing, has fueled an intensive research on altering the cascade of events that accompany wound healing. Translating such knowledge could provide immense opportunities in developing better skin equivalents for basic research and clinical therapeutic endeavors.

5.2. Roles of fibroblasts in scarless healing of the oral mucosa

Although dermal and oral mucosal wounds heal through similar stages of hemostasis, inflammation, proliferation, and remodeling, oral mucosal wounds tend to heal at a faster pace and, similar to fetal wounds, display no or minimal scarring (Szpaderska et al., 2003, 2005). While both oral and dermal fibroblast sub-populations are part of the greater adult fibroblast compartment, they exhibit significant phenotypic and functional differences that contribute to the differential healing outcomes observed in these sites. Of note, oral mucosal fibroblasts originate from the embryonic neural crest and dermal fibroblasts from the mesoderm (Xu et al., 2013); a difference that is carried into the adult by means of distinct expression profiles of the embryonic pattern-related HOX genes in the two populations (Chang et al., 2002; Ebisawa et al., 2011). Recently, Rinkevich et al. (2015), demonstrated the persistence of two distinct embryonic fibroblast traits: *Wnt1*-positive and negative ($Wnt1^{+}/Wnt1^{-}$) fibroblasts in oral mucosa, and $En1^{+}/En1^{-}$ fibroblasts in dorsal skin of adult mice. Both $Wnt1^{+}$ and $En1^{+}$ fibroblast lineage contribute to the bulk of ECM deposition in the oral mucosa and skin, respectively. However, reciprocal transplantation of $Wnt1^{+}$ and $En1^{+}$ fibroblasts into skin and oral mucosa, respectively, show significantly different scar architecture and reduced scarring by $Wnt1^{+}$ compared to $En1^{+}$ fibroblasts. These findings indicate the site-specific differences in oral and dermal wound healing, are due to cell-intrinsic differences rather than the local environment.

Apparently the fibroblast population of the adult oral mucosa has more in common with fetal fibroblasts than with their dermal counterparts: human oral mucosal fibroblasts proliferate more, repopulate wounds more rapidly and are “replicatively younger” than dermal fibroblasts (Enoch et al., 2009, 2010; Mah et al., 2014). In addition to differences in the phenotype of the fibroblasts, differences in ECM and ECM-modulating factors also play a role in the scarless healing of oral wounds. Cultured oral mucosal fibroblasts express higher levels of MMP-2 and MMP-3, and correspondingly lower levels of their antagonists, TIMP-1 and TIMP-2, compared to dermal fibroblasts (Enoch et al., 2010; Mah et al., 2014; McKeown et al., 2007; Stephens et al., 2001a). The high ratio of MMPs to TIMPs in oral mucosal wounds is comparable to that in early gestational wounds (Dang et al., 2003a). Tenascin-C is an important regulator of cell proliferation and migration during wound healing and its basal level of expression is markedly higher in oral palatal mucosa than in skin; wounding of the mammalian oral mucosa leads to prolonged tenascin-C expression, similar to fetal wounds (Whitby et al., 1991; Wong et al., 2009). Hence, an increased and prolonged expression of tenascin-C may play a role in accelerated wound healing. Another ECM molecule that plays a role in wound healing is

HA, the synthesis of which being dependent on the activity of the different isoforms of hyaluronan synthase (HAS). High-molecular weight HA is synthesized with the aid of HAS-1 and HAS-2 isoforms, while HAS-3 isoform synthesize low-molecular weight HA. In contrast to dermal fibroblasts, human oral fibroblasts exhibit differential expression of HAS subtypes and production of HA (Yamada et al., 2004). Exposure of cultured human oral mucosal fibroblasts to IL-1β or epidermal growth factor (EGF) results in expression of HAS-3 transcripts, while dermal fibroblasts express HAS-1 transcripts (Yamada et al., 2004). Similarly, *in vitro* exposure to TGFβ1 results in induction of HAS-1 expression in dermal fibroblasts but not in oral mucosal fibroblasts (Meran et al., 2007). Though both the fibroblast subtypes express HAS-2 isoforms, exposure to TGFβ1 upregulates HAS-2 expression in dermal fibroblasts, while the human oral fibroblasts display a down-regulation (Meran et al., 2007). These findings suggest the appropriate expression of HAS isoforms and the synthesis of appropriate HA molecules might play a role in the wound healing response and in skin tissue engineering.

In addition to differences in ECM remodeling, oral mucosal fibroblasts display differences in production and response to various growth factors and cytokines. For instance, cultured oral mucosal fibroblasts express significantly higher amounts of KGF and HGF (Okazaki et al., 2002; Shannon et al., 2006; Stephens et al., 2001b). The multifunctional cytokine TGFβ isoforms play a pivotal role in wound healing through its effect on fibroblasts. In contrast to dermal wounds, oral mucosal wounds exhibit significantly lower levels of profibrotic TGFβ1 and higher levels of antifibrotic TGFβ3 (Eslami et al., 2009; McKeown et al., 2007). Interestingly, TGFβ3 persists for a prolonged period in oral mucosal wounds, and TGFβ1 suppresses proliferation of oral fibroblasts while it activates proliferation of dermal fibroblasts *in vitro* (Meran et al., 2008). Additionally, cultured human oral fibroblasts express less α-SMA upon stimulation with TGFβ1 compared to dermal fibroblasts (Lygoe et al., 2007; Meran et al., 2007), indicating lower responsiveness of oral fibroblasts to TGFβ1 that favors a scarless healing. *In vitro* studies demonstrate that oral mucosal fibroblasts produce lesser contraction of collagen gels compared to dermal fibroblasts both in the presence or absence of TGFβ1; but the contraction occurs earlier in the presence of oral mucosal fibroblasts similar to fetal fibroblasts (Lee and Eun, 1999; McKeown et al., 2007; Shannon et al., 2006). Secondly, the contractile ability of the oral and fetal fibroblasts exists in spite the absence of α-SMA expression. The lower contractile potency of oral fibroblasts may be one of the factors that can explain the faster healing with lower propensity for scar formation in oral wounds. Hence, the ratio of TGFβ3 to TGFβ1 and the response of the fibroblasts to TGFβ1 could be used as screening criteria to select different fibroblast phenotypes and also as a predictive tool for contraction of fibroblasts populated collagen-based organotypic cultures of skin.

Organotypic oral mucosa models have been developed using cocultures of oral mucosal fibroblasts and oral keratinocytes on various dermal scaffolds and de-epidermized mucosa (Costea et al., 2003, 2005; Dongari-Bagtzoglou and Kashleva, 2006; Kriegebaum et al., 2012; Lukandu et al., 2010; MacNeil et al., 2011; Moharamzadeh et al., 2012). Similar to *in vivo* heterotopic recombination studies described earlier, *in vitro* homotypic organotypic cultures using human fibroblasts and keratinocytes from skin and oral mucosa show the resemblance of engineered homotypic epithelial tissues to parent tissues (skin/oral mucosa), but the heterotypic cultures of oral fibroblasts and skin keratinocytes resulted in formation of epithelium similar to oral mucosa (Chinnathambi et al., 2003). While the fetal-like phenotype and high accessibility of oral mucosal fibroblasts (as opposed to restricted access to fetal fibroblasts) are attractive for tissue engineering purposes, the inherent epigenetic memory within these fibroblasts precludes their use for skin tissue engineering. However, the insights learnt from the phenotype of oral fibroblasts could be used to select or genetically engineer dermal fibroblasts favorable for superior organotypic skin cultures. Secondly, deeper understanding of the potential mechanisms contributing to the scarless phenotype of oral mucosal wounds is needed to understand the mechanisms operating in the dermal and oral wounds.

5.3. *Involvement of fibroblasts in keloids and hypertrophic scarring*

While fetal and oral mucosal wounds exist at one end of the wound healing spectrum, hypertrophic scars and keloids represent the other end: these pathological scarring conditions occur due to an aberrant fibroblastic response following a cutaneous injury. The scars themselves are characterized by hyperproliferation of the resident wound fibroblasts, excessive ECM production (especially collagen type-I), persistence of myofibroblasts, and increased expression of α-SMA and various cytokines including TGFβ1 (Jumper et al., 2015; Lee et al., 2004). Histopathology reveals that the arrangement of fibroblasts and the deposition of collagen are haphazard in these lesions (Lee et al., 2004).

Both genetic and environmental factors are involved in susceptibility to pathological scarring, but it has been proposed that abnormalities in epidermal-dermal crosstalk at the wound site also play a role. Lesional keratinocytes and fibroblasts are significantly different in comparison to non-lesional skin; keloid and hypertrophic scar fibroblasts express higher levels of α-SMA *in situ* (Ehrlich et al., 1994; Machesney et al., 1998), while lesional keratinocytes in the basal layer of the epidermis show higher expression of activin-A and follistatin (Mukhopadhyay et al., 2007). Moreover, lesional keratinocytes seem to be in a prolonged state of activation, indicated by expression of K6, K16 and K17, which is accompanied by premature expression of filaggrin (Machesney et al., 1998). Similar differences in the phenotypes of lesional and non-lesional fibroblasts and keratinocytes are also observed *in vitro* and are therefore relatively well-defined. Compared to normal fibroblasts, keloid-derived fibroblasts in monolayer cultures show increased expression of collagen type I, activin-A, follistatin, α-SMA, thrombospondin, and matrix remodeling factors including MMP-1 and -2, and TIMP1 (Chipev et al., 2000; Fujiwara et al., 2005; Mukhopadhyay et al., 2007; Phan et al., 2002). Studies suggests that the cytokine TGFβ1 plays a role in regulating the production of ECM and ECM remodeling factors (Fujiwara et al., 2005), and accordingly, the addition of neutralizing anti-TGFβ antibodies to cultures can partially revert the phenotype of keloid fibroblasts (Younai et al., 1996). Compared to cultures derived from normal skin, cocultures of keratinocytes and fibroblasts from keloids exhibit higher expression of TGFβ1, TGFβ3 and TGFβ receptor 1 in the keratinocytes, associated with higher expression of TGFβ1, TGFβ2, TGFβ receptor 1 and Smad2 by fibroblasts (Xia et al., 2004). Studies have also indicated the roles of keloid/hypertrophic scar keratinocyte-derived growth factors, including platelet-derived growth factor and insulin-like growth factors, in driving the proliferation of dermal fibroblasts and synthesis of dermal matrix components that lead to scar formation (Funayama et al., 2003; Lim et al., 2001, 2002; Mukhopadhyay et al., 2005; Niessen et al., 2001; Phan et al., 2002, 2003a; Robb et al., 1987). However, various questions like the key events involved in aberrant change in the normal wound healing mechanisms, the timing of these events, factors that trigger the phenotypic switch, factors that drive or maintain the activated state of fibroblasts, why certain areas of the body are more susceptible, and the process that aid the extension of keloid scars beyond the original wound margin are poorly understood.

The opioid receptor system is another important component in epidermal homeostasis and wound healing (Bigliardi-Qi et al., 1999, 2000; Bigliardi and Bigliardi-Qi, 2014; Bigliardi et al., 1998, 2009).

Hypertrophic scar tissue as well as keratinocytes and fibroblasts cultured from hypertrophic scars express higher levels of all three opioid receptors (mu-, delta- and kappa-, or MOPr, DOPr, KOPr) than their counterparts in non-scarred skin (Cheng et al., 2008) suggesting a link between the opioid receptor system and hypertrophic scars. Treatment of skin organ cultures from normal human skin with β-endorphin resulted in upregulation of TGFβ receptor II and cytokeratin K16 in the epidermis (Bigliardi-Qi et al., 2000), which could be highly relevant in the case of hypertrophic scarring, as discussed above. *In vitro* cell culture studies show that ectoderm-derived cells (keratinocytes and melanocytes) express higher levels of MOPr than DOPr, while the mesoderm-derived cells (fibroblasts and dermal papilla) express higher levels of DOPr than MOPr (Bigliardi et al., 2009). We have previously reported MOPr and DOPr knockouts are associated with epidermal atrophy, thickening of the epidermis at the wound edge and delayed wound healing response (Bigliardi-Qi et al., 2006, 2007); while KOPr knockout mice exhibit epidermis of normal thickness (Bigliardi-Qi et al., 2007) suggesting a role of the opioid receptor system for keratinocyte migration and differentiation. Organotypic cultures of DOPr-overexpressing N/TERT-1 cells develop an atrophic epithelium lacking keratin 10 expression (marker of early differentiation) compared to controls suggesting the role of DOPr on epidermal homeostasis and regeneration (Neumann et al., 2015). Though the role of opioid receptors on keratinocytes has become to be acknowledged, their role in different populations of dermal fibroblasts and in hypertrophic scars/keloids has yet to be probed.

As few animal models of hypertrophic scarring and keloids are available (Estrem et al., 1987; Wang et al., 2011; Zhu et al., 2003), the organotypic coculture model is a valuable tool in dissecting the pathogenesis underlying these scars. Butler et al. observed increased collagen deposition, denser organization of α-SMA fibers, and greater dermal contracture in organotypic skin cultures derived from keloid fibroblasts and keratinocytes compared to those from normal skin (Butler et al., 2008; Chiu et al., 2005). Moreover, lesional fibroblasts demonstrated increased contraction of both fibrin and collagen gels (Phan et al., 2003b; Younai et al., 1996) indicating a more contractile phenotype of keloid fibroblasts. Further, cultured keloid fibroblasts seem to generate higher amounts of intracellular photosensitizer (protoporphyrin IX) and reactive oxygen species, and revealed higher cytotoxicity compared to normal fibroblasts after photodynamic therapy and electrical stimulation (Sebastian et al., 2011). Thus organotypic cultures have also been used to comprehend the effects of treatments on scarred skin (Chiu et al., 2005; Lee et al., 2013). Exposure of cocultures of neonatal, adult and keloid-derived fibroblasts and keratinocytes to various doses of photodynamic therapy revealed that keloid fibroblasts respond differently than those from normal skin (Chiu et al., 2005). Furthermore, recent investigations suggest the existence of heterogeneity among fibroblasts within the keloid lesion. A recent study engrafted organotypic skin derived from keloid keratinocytes and fibroblasts onto athymic mice, and found differences in scar characteristics between superficial and deep keloid-fibroblasts (Supp et al., 2012). The authors hypothesized that the superficial (papillary) and deep (reticular) keloid fibroblasts contribute differentially towards the formation of the scar tissue. Deep keloid fibroblasts are proposed to cause thickening of the scar due to an imbalance between ECM production and degradation, while the superficial keloid fibroblasts play a role in increasing the surface area of the scar tissue. These keloid fibroblast phenotypes are not only heterogeneous in terms of their functional role and response to cytokines or growth factors, but also to immunomodulatory (Russell et al., 1995) and photodynamic (Mendoza et al., 2012) therapies. Keloid fibroblasts seem to differentially respond to photodynamic therapy depending on the location of the fibroblasts within the lesion (Mendoza et al., 2012). In this study, the authors demonstrated that keloid fibroblasts from the core of the keloid lesion displayed higher cytotoxicity to photodynamic therapy compared to those from superficial dermis and margins of the lesion suggesting a site-targeted therapy.

In addition to intrinsic differences in the fibroblast phenotypes, the local microenvironment in particular the composition of the ECM, may modulate fibroblast behavior. For instance, in the deeper dermis the ECM contains lower levels of anti-fibrotic molecules such as decorin and fibromodulin compared to the superficial dermis (Honardoust et al., 2011, 2012a,b; Varkey et al., 2011). Hence, both heterogeneity in the fibroblast subpopulations and their microenvironment play an important role in the pathogenesis of keloids and hypertrophic scars. From a skin tissue engineering point of view, appropriate expression of fibrotic and anti-fibrotic factors by the fibroblasts and the surrounding ECM (*i.e.*, dermal equivalent) should be given due consideration.

6. Fibroblasts in engineered medical skin replacements

Ideal tissue engineered skin replacements for clinical use must be able to support complete reconstruction of physiological skin without any scar tissue, prevent fluid and heat loss, be non-toxic, biocompatible, free of pathogens, must lack immunogenicity, provide a barrier to micro-organisms and lack the potential for tumorogenecity. Yet they have to be cost-effective, easily available, user-friendly and have long shelf life (MacNeil, 2007). In addition to restoration of epidermal and dermal layers, complete reconstruction of various anatomical aspects of skin includes reinstitution of skin pigmentation, adnexal structures, vascularity, and innervation. A number of strategies have been explored in the past few decades to achieve the goals of skin tissue engineering that are excellently reviewed elsewhere (Kamel et al., 2013; Metcalfe and Ferguson, 2007; Shevchenko et al., 2010). Currently, split-thickness autologous skin grafts are considered the gold-standard for treatment of full-thickness dermal injuries (Adzick and Longaker, 1991). These skin grafts consist of epidermis and a superficial part of the dermis harvested using a dermatome from undamaged, healthy donor site (undamaged, healthy area). Various skin equivalents are commercially available for treatment of skin loss which are classified as acellular or cellular; biological (autologous, allogeneic, xenogeneic) or synthetic (biodegradable, non-biodegradable); epidermal, dermal or dermo-epidermal (full-thickness) skin substitutes (listed in Tables 4–6) and are excellently reviewed elsewhere (MacNeil, 2007; Patel and Fisher, 2008; Shevchenko et al., 2010). Currently, dermo-epidermal skin substitutes are the most advanced and sophisticated skin replacement that consist of allogeneic keratinocytes seeded onto a dermal scaffold with or without fibroblasts. In spite of the presence of both the dermal and epidermal compartments, these skin substitutes act as temporary wound dressings that are biologically active through the provision of cells, growth factors, cytokines and ECM that aids the recruitment of host cells and eventually healing of the wound area (Supp and Boyce, 2005). The vast variety of skin substitutes available on the market has contributed to the improvement in the management of burns, chronic dermal wounds, immunological and congenital skin disorders. However, none of the currently available skin substitutes fulfill all the above-mentioned criteria nor are they capable of replacing all the anatomical and functional properties of the native skin (Shevchenko et al., 2010).

Various challenges regarding the clinical use of existing commercially available skin substitutes include issues related to scarring, deficient vascularization, absence of adnexal structures of the skin, delay during the cell culture process, allogeneic cell source, immunogenicity, mechanical and handling properties, safety issues and high production costs (Metcalfe and Ferguson, 2007). Scarring is one of main clinical challenges that may involve the whole

Table 4
Commercially available epidermal equivalents for clinical applications.

Brand name	Manufacturer	Cells	Cell source	Cell seeding	Scaffold material	Advantages	Disadvantages
Bioseed-S	BioTissue Technlogies (Germany)	Subconfluent suspension of cultured keratinocytes	Autologous	*in vitro*	Fibrin sealant	Permanent, better handling, improved cell attachment, haemostasis and wound healing. Little risk of rejection	Long culture time, limited to partial-thickness wounds
CellSpray	Clinical Cell Culture (C3, Australia)	Subconfluent suspension of cultured keratinocytes	Autologous	*in vitro*	–	Reduced culture time, better handling, permanent, little risk of rejection	Limited to partial-thickness wounds
EPIBASE	Laboratories Genevrier (France)	Confluent sheet of cultured keratinocytes	Autologous	*in vitro*	–		
Epicel	Genzyme Biosurgery (USA)	Confluent sheet of cultured keratinocytes	Autologous	*in vitro*	–	Permanent, large area of wound coverage, little risk of rejection	Long culture time (atleast 3 weeks), fragile confluent sheets, susceptible to blistering (post-grafting)
Epidex	Modex Therapeutics (Switzerland)	Confluent sheet of cultured keratinocytes	Autologous; from outer root sheath of scalp hair follicles	*in vitro*	–	Permanent, little risk of rejection, higher proliferative capacity. Can be cryopreserved for repeat applications	Long culture time (atleast 5–6 weeks), fragile confluent sheets
Laserskin or Vivoderm	Fidia Advanced Biopolymers (Italy) (Convatec)	Confluent sheet of cultured keratinocytes	Autologous	*in vitro*	Recombinant hyaluronic acid membrane	Less fragile delivery system, Hyaluronic acid improves mechanical stability, little risk of rejection	Long culture time (atleast 3 weeks)
MySkin	CellTran Ltd (UK)	Subconfluent sheet of cultured keratinocytes	Autologous	*in vitro*	Silicone support layer with a plasma-polymerized surface	Silicone support layer encourages keratinocyte attachment and proliferation. Improved and stable delivery platform. Can be cryopreserved for repeat applications	Long culture time (atleast 2 weeks). Repeated application required for optimal clinical outcome

replaced tissue and/or the graft margins which in turn results in a skin that is functionally, mechanically and/or esthetically inferior to native skin. Additionally, scar tissue is not amenable for regeneration of skin appendages. To address this problem, the next generation skin substitutes should possess structural and functional properties of native skin and anti-scarring strategies should aid the integration with the host tissue without any scarring.

As previously discussed fibroblasts and their surrounding ECM play an important role in the scarring process. Fibroblasts currently in use for engineering skin tissue replacements are: autologous or allogeneic, primary cells or progenitors. Autologous fibroblasts are advantageous as they lack risk of rejection and cross contamination with blood-borne and/or xenogeneic pathogens. However, they are not readily available and may not be available in sufficient cell numbers especially at old age and from patients with extensive burns. In contrast, allogeneic cells could be obtained from young donors, expanded in culture to sufficient quantities, cryopreserved and thus amenable for off-the-shelf availability. Early studies comparing dermal substitutes populated with autologous or allogeneic fibroblasts revealed better restoration of dermis with minimal scar formation when autologous fibroblasts are used (Lamme et al., 2002; Morimoto et al., 2005). Studies on immunological tolerance of allogeneic cells on the recipient and survival of donor cells in the host show varying findings. In a short-term study on guinea pig (Morimoto et al., 2005) survival of allogeneic fibroblasts was found in the host up to three-weeks, while studies in pig (Price et al., 2004) and humans (Kolokol'chikova et al., 2001) reported absence of allogeneic fibroblasts after 1 week. Certain long-term studies demonstrate the presence of proliferating allogeneic fibroblasts and host immunogenic tolerance up to two months (Eaglstein et al., 1999; Griffiths et al., 2004; Hebda and Dohar, 1999; Sandulache et al., 2003). In contrast, clinical studies using a bilayered skin substitute composed of allogeneic keratinocytes and fibroblasts in a collagen gel demonstrated the absence of DNA of donor cells beyond 4–6 weeks (Griffiths et al., 2004; Hu et al., 2006; Phillips et al., 2002). These clinical studies also did not reveal any evidence of immunological rejection of the graft which is believed to be due to lack of expression of major histocompatibility complex (MHC-class II) antigens (Theobald et al., 1993) and absence of antigen-presenting cells like Langerhan's cells following serial passages of allogeneic fibroblasts and keratinocytes (Phillips et al., 2002). Further, clinical studies demonstrate that allogeneic fibroblast populated dermal substitutes with or without keratinocytes in chronic venous ulcers, diabetic foot ulcers and pressure ulcers result in significant healing compared to conventional treatment alone (Brem et al., 2000; Falanga et al., 1998; Jones and Nelson, 2005; Marston et al., 2003). These studies demonstrate the immunological safety in using allogeneic fibroblasts and their

Table 5
Commercially available dermal equivalents for clinical applications.

Brand name	Manufacturer	Cells	Cell source	Cell seeding	Scaffold material	Advantages	Disadvantages
Alloderm	LifeCell Corporation (USA)	–	–	*in vivo*	Allogeneic; Human acellular lyophilized dermis	Permanent; Preserved native human ECM; Reduced antigenic components due to processing; Off-the-shelf	Potential risk of disease transmission
Allopatch HD	Conmed (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis	Permanent; Preserved native human ECM; Reduced antigenic components due to processing; Off-the-shelf	Potential risk of disease transmission
AlloSkin RT	AlloSource (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis (irradiated); Meshed	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Pliable and stretchable; Off-the-shelf	Potential risk of disease transmission
Cortiva Allograft Dermis	RTI Surgical (USA)	–	–	*in vivo*	Allogeneic; Human non-crosslinked, acellular dermis (irradiated)	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
DermACELL	LifeNet Health (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
Dermagraft	Organogenesis Inc (USA) (Advanced BioHealing Inc, USA)	Cultured Human dermal fibroblasts	Allogeneic	*in vitro*	ECM-coated polyglactin mesh; ECM derived from cultured human dermal fibroblasts	ECM components and growth factors secreted by fibroblasts aid wound healing; Bioabsorbable	Potential risk of disease transmission; Temporary
Flex HD	Ethicon (USA)	–	–	*in vitro*	Allogeneic; Human acellular dermis	Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
Fortiva Porcine Dermis	RTI Surgical (USA)	-	-	*in vivo*	Xenogeneic; Porcine non-crosslinked acellular dermis	Preserved native ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
GammaGraft	Promethean LifeSciences Inc. (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis (irradiated)	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Pliable and stretchable; Off-the-shelf.	Potential risk of disease transmission
GraftJacket	Wright Medical Technology (USA)	–	–	*in vivo*	Allogeneic; Human acellular meshed lyophilized dermis	Permanent; Preserved native human ECM; Reduced antigenic components due to processing; Convenient application as it is pre-meshed; Successful in full-thickness wounds; Off-the-shelf.	Potential risk of disease transmission
Hyalomatrix PA/Hyalofill	Anika Therapeutics (USA) (Fidia Advanced Biopolymers, Italy)	–	–	*in vivo*	Synthetic; Hyaluronic acid-based	Semi-permanent; No animal or allogeneic human-derived components	–
Integra Dermal Regeneration Template	Integra NeuroSciences (USA)	–	–	*in vivo*	Xenogeneic and synthetic (Polysiloxane); Bovine cross-linked collagen and glycosaminoglycans	Semi-permanent (epidermal equivalent replaced with autograft after 2 weeks); Polysiloxane membrane controls bacterial contamination and water loss; Encourages fibroblast and keratinocyte ingrowth; Off-the-shelf	Bovine origin; Potential risk of disease transmission; Longer time (∼3 weeks) required for expanding dermal autograft

Matriderm	MedSkin Solutions Dr Suwelack AG (Germany)	–	–	*in vivo*	Xenogeneic; Bovine acellular lyophilized dermis (coated with α-elastin hydrolysate)	Permanent; Preserved native collagen matrix; Reduced antigenic components due to processing; Successful in full-thickness burns; Off-the-shelf	Bovine origin; Potential risk of disease transmission
Matrix HD/ Matrix HD Fenestrated	RTI Surgical (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis; Meshed (Fenestrated)	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
MemoDerm	Memometal Inc. (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis (irradiated)	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
OASIS Wound Matrix	Cook Biotech Inc	–	–	*in vivo*	Xenogeneic; Porcine acellular lyophilized submucosa of small intestine	Permanent; Preserved native collagen matrix; Reduced antigenic components due to processing; Off-the-shelf	Porcine origin; Potential risk of disease transmission
Pelnac (Standard/ Fortified)	Gunze Ltd. (Japan)	–	–	*in vivo*	Xenogeneic and synthetic (silicone membrane); Porcine lyophilized cross-linked collagen	Semi-permanent; Silicone membrane controls bacterial contamination and water loss; Long shelf life; Off-the-shelf	Porcine origin; Potential risk of disease transmission; Insufficient revascularization
Permacol	Covidien (UK) (Tissue Science Laboratories, UK)	–	–	*in vivo*	Xenogeneic; Porcine acellular diisocyanite cross-linked dermal matrix	Permanent; Reduced antigenic components due to processing; Off-the-shelf	Porcine origin; Potential risk of disease transmission
ProHeal	MedSkin Solutions Dr Suwelack AG (Germany)	–	–	*in vivo*	Xenogeneic; Bovine acellular native collagen	Permanent; Preserved native collagen matrix; Reduced antigenic components due to processing; Successful in full-thickness burns; Off-the-shelf.	Bovine origin; Potential risk of disease transmission.
Puros Dermis	Zimmer Dental (USA)	–	–	*in vivo*	Allogeneic; Human acellular dermis (irradiated)	Preserved native human ECM; Reduced antigenic components due to processing; Room temperature storage; Off-the-shelf	Potential risk of disease transmission
SureDerm	Hans Biomed Corporation (Korea)	–	–	*in vivo*	Allogeneic; Human acellular lyophilized dermis	Permanent; Preserved native human ECM; Reduced antigenic components due to processing; Successful in hypertrophic scar revision and burn wounds; Off-the-shelf	Potential risk of disease transmission; requires 10 min rehydration before application
Terudermis	Olympus Terumo Biomaterial Corp., (Japan)	–	–	*in vivo*	Xenogeneic and synthetic (silicone membrane); Bovine heat-denatured fibrillar atelocollagen	Semi-permanent; Silicone membrane controls bacterial contamination and water loss; Successful in treatment of deep burns; Silicone membrane peels off naturally; Off-the-shelf	Bovine origin; Potential risk of disease transmission
Transcyte (Dermagraft TC)	Advanced Tissue Sciences (USA) (Advanced BioHealing Inc, USA)	Cultured Human dermal fibroblasts	Allogeneic	*in vitro*	ECM-coated nylon mesh; ECM derived from cultured human dermal fibroblasts	ECM components and growth factors secreted by fibroblasts aid wound healing	Allogeneic; Potential risk of disease transmission; Temporary; Nylon mesh is not biodegradable

Table 6
Dermo-epidermal composite skin equivalents for clinical applications.

Brand name	Manufacturer	Cells	Cell source	Cell seeding	Scaffold material	Advantages	Disadvantages
AlloSkin	AlloSource (USA)	Keratinocytes, fibroblasts	Allogeneic; Cadaveric; Cryopreserved	-	Cadaveric human skin; Meshed	Full thickness skin graft; Delivers ECM, components, cytokines and growth factor.	Potential risk of disease transmission
Apligraf	Organogenesis Inc (USA)	Keratinocytes, fibroblasts	Allogeneic; Human neonatal foreskin-derived	*in vitro*	Xenogeneic; bovine type I collagen	Full thickness skin graft; Graft uptake similar to autografts; Delivers ECM, components, cytokines and growth factors	Very short shelf life (~5 days); Temporary bioactive dressing; needs delicate handling; Potential risk of disease transmission; High cost
ExpressGraft	Stratatech Corp.(USA)	Keratinocytes, fibroblasts	Allogeneic; Keratinocytes: Genetically modified immortalized human keratinocyte progenitor cell line (NIKS) Fibroblasts: Human dermal fibroblasts	*in vitro*	Type I collagen (non-bovine) (proprietary)	Full thickness skin graft	Not commercially available (Clinical trial phase); use of allogeneic, genetically modified, immortalized cell lines
OrCel	Forticell Bioscience, Inc.(USA) (Ortec International)	Keratinocytes, fibroblasts	Allogeneic; Human neonatal foreskin-derived	*in vitro*	Xenogeneic; bovine type I collagen sponge	Full thickness skin graft; Graft uptake similar to autografts; Delivers ECM, components, cytokines and growth factors; Cryopreserved	Needs delicate handling; Potential risk of disease transmission; High cost
PermaDerm	Regenicin	Keratinocytes, fibroblasts	Autologous	*in vitro*	Bovine type I collagen	Full thickness skin graft; Autologous	Not commercially available (Clinical trial phase); Long culture time
PolyActive	HC Implants BV (Netherlands)	Keratinocytes, fibroblasts	Autologous	*in vitro*	Synthetic PolyActive matrix (polyethylene oxide terephthalate and polybutylene terephthalate)	Full thickness skin graft; Autologous	Not commercially available (Clinical trial phase); Long culture time; Primarily used for bone reconstruction; non-biodegradable
StrataGraft	Stratatech Corp.(USA)	Keratinocytes, fibroblasts	Allogeneic; Keratinocytes: immortalized human keratinocyte progenitor cell line (NIKS) Fibroblasts: Human dermal fibroblasts	*in vitro*	Type I collagen (non-bovine) (proprietary)	Full thickness skin graft	Not commercially available (Clinical trial phase); use of allogeneic immortalized cell lines
TheraSkin	Soulble Systems (USA)	Keratinocytes, fibroblasts	Allogeneic; Cadaveric; Cryopreserved	–	Cadaveric human skin; Meshed	Full thickness skin graft; Delivers ECM, components, cytokines and growth factors	Potential risk of disease transmission

role in wound healing in spite of their disappearance over a period of few weeks. However, the wounds healed with formation of scar tissue indicating a suboptimal regeneration of the skin.

To modulate the scar tissue formation following grafting with tissue engineered skin substitutes various strategies have been pursued. A study using porcine full-thickness wound model, demonstrated improvement in wound healing and modulation of scar tissue with higher number of fibroblasts present in the dermal substitute at the moment of transplantation (Lamme et al., 2000). Based on the understanding of cellular and molecular mechanisms of scarring and scarless fetal wounds, many companies are developing novel pharmaceutical compounds that prophylactically prevent scar formation (excellently reviewed elsewhere, Occleston et al., 2008; Rhett et al., 2008). A promising pharmaceutical candidate avotermin (recombinant human TGFβ3) for prevention of that was developed based on the finding that scarless fetal and oral wounds possess lower levels of profibrotic factors (like TGFβ1, TGFβ2, and PDGF) and higher levels of anti-fibrotic molecules (like TGFβ3) (Occleston et al., 2008, 2011). Though phase I/II trials were successful, avotermin failed in phase III trials and is currently discontinued. Similarly, another product based on recombinant human IL-10 (ilodecakin) for scar prevention was discontinued after phase II trials. Studies investigating the development of novel skin substitutes with appropriate combination of fibroblasts and ECM that favor

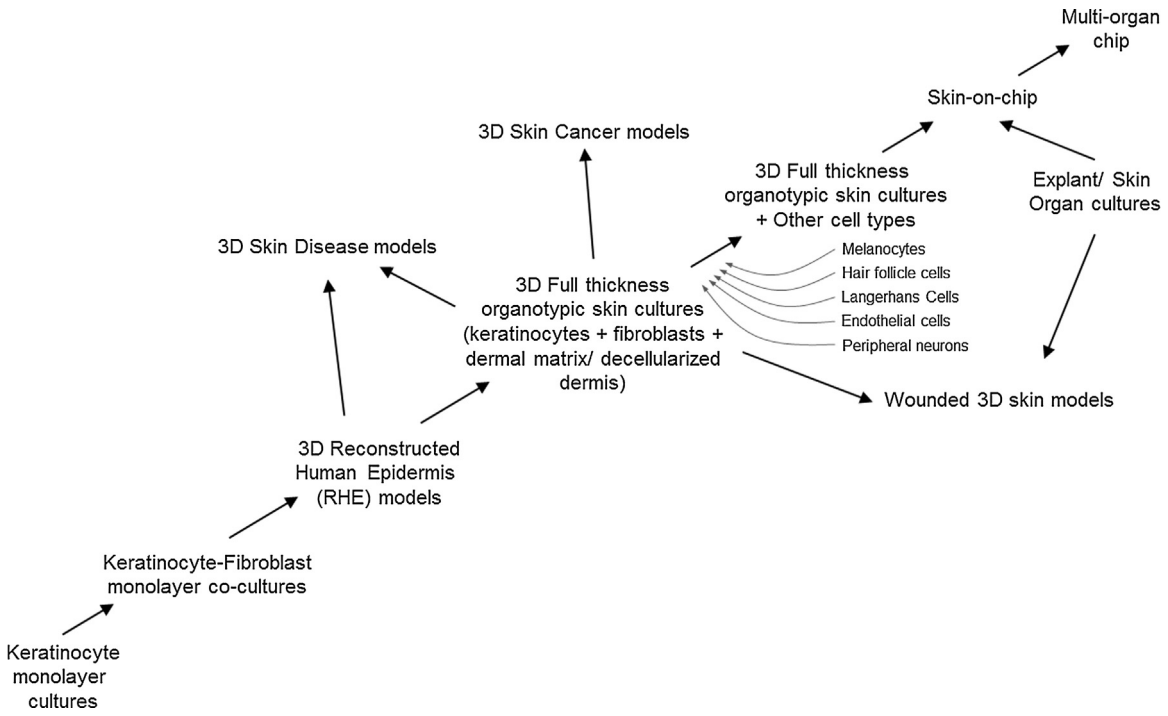

Fig. 6. Flow chart schematically represents the development of organotypic skin cultures from a simple monolayer cultures to keratinocyte-based reconstructed human epidermis models to complex full-thickness models. These full-thickness skin culture models gave birth to various skin disease, skin cancer, skin wound and more complex skin culture models. Currently, these models are being miniaturized and incorporated into microfluidic chips for skin-on-chip and multi-organ chip platforms.

scar-free healing and anti-fibrotic strategies are needed to enhance integration with the host tissue.

Similar to TGFβ signaling, differential expression and balance of FGF and VEGF isoforms and receptors are hypothesized to play a role in scarless fetal wound healing (Colwell et al., 2005; Dang et al., 2003b). Studies demonstrate acceleration of tissue regeneration by a dermal substitute incorporated with collagen/gelatin-FGF2 microspheres through accelerated fibroblast proliferation and angiogenesis in a dose-dependent manner (Kanda et al., 2012; Kawai et al., 2000). Delayed or insufficient angiogenesis at the wound site play a role in chronic wounds, resulting in delayed or failure of graft uptake in deep burns. A study on fibrin-based dermal substitutes cultured with human fibroblasts and keratinocytes demonstrated higher levels of VEGF secretion, improved regeneration of epidermal structures and migration of endothelial cells compared to collagen-based dermal substitutes (Hojo et al., 2003). Coculture experiments using dermal fibroblasts and endothelial cells demonstrate that papillary fibroblasts are more permissive for supporting differentiation of endothelial cells compared to reticular fibroblasts (Sorrell et al., 2008). Delayed or insufficient angiogenesis at the wound site play a role in chronic wounds, and delayed or failure of graft uptake in deep burns. Hence, the phenotype of fibroblasts at the wound bed may play a crucial role in wound healing. Engineering dermal-epidermal skin substitutes with angiogenesis-permissive phenotype of fibroblasts must be given due consideration. Recently, there are efforts at engineering vascularized skin equivalents using tri-culture of fibroblasts, endothelial cells and keratinocytes (Black et al., 1998; Chan et al., 2012; Marino et al., 2014). The phenotype of all these cells including fibroblasts might need to be considered in the development of next generation of skin equivalents.

Ultimate tissue engineering goal would be to combine various growth factors, cytokines, transcription factors, ECM components, pharmaceutical compounds and cells based on lessons learned from embryonic and adult wound healing to develop clinically relevant novel skin substitutes for complete skin regeneration. In spite of growing evidence on fibroblast heterogeneity and their role in epidermal morphogenesis and wound healing, none of the commercially available tissue engineered skin replacements and clinical studies seem to have addressed it. A skin substitute with optimal combination of dermal fibroblasts and their ECM reflecting the physiological heterogeneity of fibroblast populations and their microenvironment is yet to be designed and investigated. Hence, it would be imperative to investigate more intensively the role of fibroblast heterogeneity for clinical applications. Furthermore, the phenotypic differences within the wound bed, differences in their behavior at different stages of the wound healing and in different diseased states needs to be investigated and taken into consideration in developing novel, therapeutic skin replacements.

7. Concluding remarks

Use of increasingly complex *in vitro* models, in particular 3D organotypic cultures mimicking features of the healthy and diseased human skin represents a valuable tool. These organotypic skin cultures have been used for the past three decades in the field of dermatology research that include basic research to understand dermal-epidermal, cell–cell and cell–matrix interactions in health and disease, as an *in vitro* tool to model healthy and diseased states, for cosmetic and pharmaceutical drug development and testing, and development of therapeutic skin replacement products. However, they have various short-comings that limit their applications. For instance, shrinkage of the dermal matrix limits their culture period and also their utility for basic and clinical research. Hence, improvements in regard to the composition of ECM of the dermal compartment, physical stability of the dermal equivalent and ability for long term cultures are needed. Additionally, the barrier properties of skin equivalents are inferior compared to native skin as assessed by transcutaneous permeability assays and composition of various lipid signatures within the stratum corneum (Asbill et al., 2000; Godin and Touitou, 2007; Thakoersing

et al., 2012, 2013). Native skin is composed of different fibroblasts phenotypes that vary in terms of density, anatomic distribution and functional aspects. As dermal-epidermal interactions play a major role in epidermal morphogenesis, reconstruction of human skin with appropriate combination of different fibroblast phenotypes within the dermal compartment might result in engineering superior skin equivalents. In addition to the mere presence of fibroblasts, factors that play a role in epidermal morphogenesis include the phenotype of fibroblasts, their density within different layers of the dermal matrix, timing of fibroblast pre-conditioning phase before the addition of keratinocytes, use of post-mitotic fibroblasts, age of the donor, effect of environmental factors like ultraviolet light and probably the effect of other cell types including melanocytes, endothelial cells and hair follicle cells.

Generally, all fibroblast phenotypes express an array of intracellular and surface markers like vimentin, fibroblast-specific protein-1 (FSP-1), CD90, CD73, CD44, CD13 (aminopeptidase N), versican, and α-SMA (Sorrell and Caplan, 2009). However, these markers are not specific to fibroblasts and hence, pose difficulties in isolation of fibroblast subpopulations. Second, there are no standards available for fibroblast densities and pre-conditioning period, probably due to differences in dermal matrices and media formulations (presence or absence of serum and growth factors). For instance, fibroblasts proliferate better under serum-containing conditions; and scaffold-based dermal constructs like nylon meshes, collagen-glycosaminoglycan sponges or self-assembled fibroblast sheets generally require longer pre-conditioning periods in comparison to native ECM-based matrices like collagen or fibrin. Future research addressing methods to identify different fibroblast phenotypes, their functional profiles, and establishing standards for their use is pertinent for development of next generation of skin equivalents.

Insights from wound healing in the fetus, oral mucosa and diseased states provide us deeper understanding on the role of fibroblasts and the surrounding microenvironment in reconstructing the lost dermal structures. In addition to the fibroblasts, the role of stem cell populations like MSCs or adipose-derived stem cells (AdSCs) in repair and regeneration is gaining importance. Further, development in the stem cell field has provided us with opportunities to explore the potentials of pluripotent stem cells that include human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Adult stem cells (Chavez-Munoz et al., 2013; Han et al., 2011; Lee et al., 2010; Tran et al., 2011) and pluripotent stem cells (Fu et al., 2010; Guenou et al., 2009; Hewitt et al., 2009; Kidwai et al., 2013; Metallo et al., 2008; Petrova et al., 2014; Selekman et al., 2013; Tan et al., 2013) are known to be capable of differentiation to various somatic lineages, including fibroblasts and keratinocytes. Further, organotypic culture of the fibroblasts and keratinocytes obtained from these stem cells have been demonstrated to possess the ability to reconstruct the dermis and epidermis (Garzon et al., 2013; Guenou et al., 2009; Itoh et al., 2013; Metallo et al., 2010; Petrova et al., 2014; Selekman et al., 2013). Due to a primitive state of these stem cells and the ability to coax their differentiation to fibroblasts, it is plausible to obtain different fibroblast phenotypes by controlling the signals that drive their differentiation. Recently, Gnedeva et al. (2015) demonstrated the ability to drive the differentiation of hESCs towards neural crest and further to dermal papilla cells that upon implantation into immunodeficient mice had the ability to induce hair formation. Similarly, it is plausible to differentiate these stem cells to fibroblasts that are phenotypically similar to fetal, papillary or reticular dermal fibroblasts. Second, it is also possible to model diseased phenotypes like genodermatoses using pluripotent stem cells as a source of skin cells. Furthermore, advances in the stem cell field opens up the potential to generate large numbers of donor-matched cells with the possibility of off-the-shelf availability that could potentially advance development of next generation of skin equivalents. These next generation organotypic skin equivalents are promising from a stand-point of basic research and industrial purposes like cosmetic/chemical/pharmaceutical product testing. However from a clinical standpoint, the use of fetal-derived cells, hESCs and iPSCs are limited by safety (tumor formation), histocompatibility, regulatory and ethical concerns. The development of novel methods for generation and differentiation of hESCs and iPSCs that address these limitations is promising.

Certainly, a complex full-thickness *in vitro* skin model resembles more closely human skin than simple monolayer cultures or cocultures of individual cells. Hence, *in vitro* models to study skin biology in health and disease have moved from simple monolayer cultures and cocultures to 3D organotypic skin equivalents. These 3D skin equivalents composed traditionally of keratinocytes and fibroblasts is becoming increasingly more complex and more close to native skin through incorporation of other skin cell types including melanocytes (Cario-Andre et al., 2007; Duval et al., 2001), Langerhans cells (Facy et al., 2004, 2005), endothelial cells (Chan et al., 2012; Marino et al., 2014; Sanchez-Munoz et al., 2015), and peripheral neurons (Blais et al., 2014). Further, addition of other cell types including cells of the hair follicle and sweat glands are currently in process. Similarly, the platform of organotypic skin cultures is extended to model various diseased conditions including psoriasis (Bernard et al., 2007, 2012; Jean et al., 2009), vitiligo (Bessou et al., 1997; Cario-Andre et al., 2007), dermatitis (Bernard et al., 2012; Gschwandtner et al., 2013; Kuchler et al., 2011; Mildner et al., 2010; van den Bogaard et al., 2013), melanoma (Li et al., 2010, 2011; Marrero and Heller, 2012; Vorsmann et al., 2013), squamous cell carcinoma (Commandeur et al., 2012; Depner et al., 2014), aging (Pageon, 2010; Pageon et al., 2007), photo aging (Bernerd and Asselineau, 2008; Bernerd et al., 2012), pressure ulcers (Bronneberg et al., 2006) and wounds (Egles et al., 2008; El Ghalbzouri et al., 2004; Smithmyer et al., 2014; van Kilsdonk et al., 2013). Recently, cosmetic giant L'Oreal has partnered with 3-D bio-printing company Organovo Holdings, Inc. to develop 3D printed skin tissue for *in vitro* cosmetic product evaluation and other advanced research applications. Furthermore, the platform for *in vitro* skin cultures is moving ahead to incorporate these skin cultures within microfluidic chips to develop skin-on-chip (Atac et al., 2013) and multiorgan-on-chip (Wagner et al., 2013) models (Fig. 6, Table 2).

In conclusion, the term 'dermal fibroblasts' is an oversimplification of a dynamic, heterogeneous population of cells that exhibit differences in terms of embryological origin, anatomical distribution and functional profiles in health and disease. We are just beginning to understand the diversity of these fibroblasts and their roles in cell–cell and cell–matrix interactions that defines the structure and organization of the skin. We believe the insights learnt from the diversity and physiology of the fibroblasts in health and diseased states could pave way in the generation of superior skin equivalents.

8. Conflict of interest statement

All authors declare that there are no conflicts of interests, no financial affiliation or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript.

9. Source of funding

The work was supported by grants (JCO 1334K00081) from Joint Council Office, A*STAR, Singapore.

References

Adzick, N.S., Longaker, M.T., 1991. Animal models for the study of fetal tissue repair. J. Surg. Res. 51, 216–222.

Adzick, N.S., Longaker, M.T., 1992. Scarless fetal healing. Therapeutic implications. Ann. Surg. 215, 3–7.

Akagi, A., Tajima, S., Ishibashi, A., Yamaguchi, N., Nagai, Y., 1999. Expression of type XVI collagen in human skin fibroblasts: enhanced expression in fibrotic skin diseases. J. Invest. Dermatol. 113, 246–250.

Alaish, S.M., Yager, D., Diegelmann, R.F., Cohen, I.K., 1994. Biology of fetal wound healing: hyaluronate receptor expression in fetal fibroblasts. J. Pediatr. Surg. 29, 1040–1043.

Ali-Bahar, M., Bauer, B., Tredget, E.E., Ghahary, A., 2004. Dermal fibroblasts from different layers of human skin are heterogeneous in expression of collagenase and types I and III procollagen mRNA. Wound Repair Regen. 12, 175–182.

Anan, T., Sonoda, T., Asada, Y., Kurata, S., Takayasu, S., 2003. Protease-activated receptor-1 (thrombin receptor) is expressed in mesenchymal portions of human hair follicle. J. Invest. Dermatol. 121, 669–673.

Arredondo, J., Hall, L.L., Ndoye, A., Nguyen, V.T., Chernyavsky, A.I., Bercovich, D., Orr-Urtreger, A., Beaudet, A.L., Grando, S.A., 2003a. Central role of fibroblast alpha3 nicotinic acetylcholine receptor in mediating cutaneous effects of nicotine. Lab. Invest. 83, 207–225, a journal of technical methods and pathology.

Arredondo, J., Nguyen, V.T., Chernyavsky, A.I., Bercovich, D., Orr-Urtreger, A., Kummer, W., Lips, K., Vetter, D.E., Grando, S.A., 2002. Central role of alpha7 nicotinic receptor in differentiation of the stratified squamous epithelium. J. Cell Biol. 159, 325–336.

Arredondo, J., Nguyen, V.T., Chernyavsky, A.I., Bercovich, D., Orr-Urtreger, A., Vetter, D.E., Grando, S.A., 2003b. Functional role of alpha7 nicotinic receptor in physiological control of cutaneous homeostasis. Life Sci. 72, 2063–2067.

Asbill, C., Kim, N., El-Kattan, A., Creek, K., Wertz, P., Michniak, B., 2000. Evaluation of a human bio-engineered skin equivalent for drug permeation studies. Pharm. Res. 17, 1092–1097.

Asselineau, D., Bernard, B.A., Bailly, C., Darmon, M., 1989. Retinoic acid improves epidermal morphogenesis. Dev. Biol. 133, 322–335.

Asselineau, D., Darmon, M., 1995. Retinoic acid provokes metaplasia of epithelium formed in vitro by adult human epidermal keratinocytes. Differentiation 58, 297–306, research in biological diversity.

Atac, B., Wagner, I., Horland, R., Lauster, R., Marx, U., Tonevitsky, A.G., Azar, R.P., Lindner, G., 2013. Skin and hair on-a-chip: in vitro skin models versus ex vivo tissue maintenance with dynamic perfusion. Lab Chip 13, 3555–3561.

Auxenfans, C., Fradette, J., Lequeux, C., Germain, L., Kinikoglu, B., Bechetoille, N., Braye, F., Auger, F.A., Damour, O., 2009. Evolution of three dimensional skin equivalent models reconstructed in vitro by tissue engineering. Eur. J. Dermatol.: EJD 19, 107–113.

Azzarone, B., Macieira-Coelho, A., 1982. Heterogeneity of the kinetics of proliferation within human skin fibroblastic cell populations. J. Cell Sci. 57, 177–187.

Bader, B.L., Smyth, N., Nedbal, S., Miosge, N., Baranowsky, A., Mokkapati, S., Murshed, M., Nischt, R., 2005. Compound genetic ablation of nidogen 1 and 2 causes basement membrane defects and perinatal lethality in mice. Mol. Cell Biol. 25, 6846–6856.

Badiavas, E.V., Abedi, M., Butmarc, J., Falanga, V., Quesenberry, P., 2003. Participation of bone marrow derived cells in cutaneous wound healing. J. Cell. Physiol. 196, 245–250.

Bahta, A.W., Farjo, N., Farjo, B., Philpott, M.P., 2008. Premature senescence of balding dermal papilla cells in vitro is associated with p16(INK4a) expression. J. Invest. Dermatol. 128, 1088–1094.

Barisic-Dujmovic, T., Boban, I., Clark, S.H., 2010. Fibroblasts/myofibroblasts that participate in cutaneous wound healing are not derived from circulating progenitor cells. J. Cell. Physiol. 222, 703–712.

Barker, C.L., McHale, M.T., Gillies, A.K., Waller, J., Pearce, D.M., Osborne, J., Hutchinson, P.E., Smith, G.M., Pringle, J.H., 2004. The development and characterization of an in vitro model of psoriasis. J. Invest. Dermatol. 123, 892–901.

Bayreuther, K., Rodemann, H.P., Francz, P.I., Maier, K., 1988a. Differentiation of fibroblast stem cells. J. Cell Sci. (Suppl. 10), 115–130.

Bayreuther, K., Rodemann, H.P., Hommel, R., Dittmann, K., Albiez, M., Francz, P.I., 1988b. Human skin fibroblasts in vitro differentiate along a terminal cell lineage. Proc. Natl. Acad. Sci. U.S.A. 85, 5112–5116.

Bell, E., Ehrlich, H.P., Buttle, D.J., Nakatsuji, T., 1981. Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science (New York, NY) 211, 1052–1054.

Bell, E., Sher, S., Hull, B., Merrill, C., Rosen, S., Chamson, A., Asselineau, D., Dubertret, L., Coulomb, B., Lapiere, C., Nusgens, B., Neveux, Y., 1983. The reconstitution of living skin. J. Invest. Dermatol. 81, 2s–10s.

Bernard, F.X., Morel, F., Camus, M., Pedretti, N., Barrault, C., Garnier, J., Lecron, J.C., 2012. Keratinocytes under fire of proinflammatory cytokines: bona fide innate immune cells involved in the physiopathology of chronic atopic dermatitis and psoriasis. J. Allergy 2012, 718725.

Bernard, G., Auger, M., Soucy, J., Pouliot, R., 2007. Physical characterization of the stratum corneum of an in vitro psoriatic skin model by ATR-FTIR and Raman spectroscopies. Biochim. Biophys. Acta 1770, 1317–1323.

Bernerd, F., Asselineau, D., 2008. An organotypic model of skin to study photodamage and photoprotection in vitro. J. Am. Acad. Dermatol. 58, S155–S159.

Bernerd, F., Marionnet, C., Duval, C., 2012. Solar ultraviolet radiation induces biological alterations in human skin in vitro: relevance of a well-balanced UVA/UVB protection. Ind. J. Dermatol. Venereol. Leprol. 78 (Suppl 1), S15–S23.

Bessou, S., Gauthier, Y., Surleve-Bazeille, J.E., Pain, C., Taieb, A., 1997. Epidermal reconstructs in vitiligo: an extrinsic factor is needed to trigger the disease. Br. J. Dermatol. 137, 890–897.

Bigliardi-Qi, M., Bigliardi, P.L., Buchner, S., Rufli, T., 1999. Characterization of mu-opiate receptor in human epidermis and keratinocytes. Ann. N.Y. Acad. Sci. 885, 368–371.

Bigliardi-Qi, M., Bigliardi, P.L., Eberle, A.N., Buchner, S., Rufli, T., 2000. beta-endorphin stimulates cytokeratin 16 expression and downregulates mu-opiate receptor expression in human epidermis. J. Invest. Dermatol. 114, 527–532.

Bigliardi-Qi, M., Gaveriaux-Ruff, C., Pfaltz, K., Bady, P., Baumann, T., Rufli, T., Kieffer, B.L., Bigliardi, P.L., 2007. Deletion of mu- and kappa-opioid receptors in mice changes epidermal hypertrophy, density of peripheral nerve endings, and itch behavior. J. Invest. Dermatol. 127, 1479–1488.

Bigliardi-Qi, M., Gaveriaux-Ruff, C., Zhou, H., Hell, C., Bady, P., Rufli, T., Kieffer, B., Bigliardi, P., 2006. Deletion of delta-opioid receptor in mice alters skin differentiation and delays wound healing. Differentiation 74, 174–185, research in biological diversity.

Bigliardi, P.L., Bigliardi-Qi, M., 2014. Peripheral Opioids. In: Carstens, E., Akiyama, T. (Eds.), Itch: Mechanisms and Treatment, Frontiers in Neuroscience. Taylor & Francis Group, London.

Bigliardi, P.L., Bigliardi-Qi, M., Buechner, S., Rufli, T., 1998. Expression of mu-opiate receptor in human epidermis and keratinocytes. J. Invest. Dermatol. 111, 297–301.

Bigliardi, P.L., Tobin, D.J., Gaveriaux-Ruff, C., Bigliardi-Qi, M., 2009. Opioids and the skin—where do we stand? Exp. Dermatol. 18, 424–430.

Black, A.F., Berthod, F., L'Heureux, N., Germain, L., Auger, F.A., 1998. In vitro reconstruction of a human capillary-like network in a tissue-engineered skin equivalent. FASEB J. 12, 1331–1340.

Blais, M., Mottier, L., Germain, M.A., Bellenfant, S., Cadau, S., Berthod, F., 2014. Sensory neurons accelerate skin reepithelialization via substance P in an innervated tissue-engineered wound healing model. Tissue Eng., A 20, 2180–2188.

Boehnke, K., Mirancea, N., Pavesio, A., Fusenig, N.E., Boukamp, P., Stark, H.J., 2007. Effects of fibroblasts and microenvironment on epidermal regeneration and tissue function in long-term skin equivalents. Eur. J. Cell Biol. 86, 731–746.

Borchers, A.H., Powell, M.B., Fusenig, N.E., Bowden, G.T., 1994. Paracrine factor and cell–cell contact-mediated induction of protease and c-ets gene expression in malignant keratinocyte/dermal fibroblast cocultures. Exp. Cell Res. 213, 143–147.

Boukamp, P., Breitkreutz, D., Stark, H.J., Fusenig, N.E., 1990. Mesenchyme-mediated and endogenous regulation of growth and differentiation of human skin keratinocytes derived from different body sites. Differentiation 44, 150–161, research in biological diversity.

Boyce, S.T., Warden, G.D., 2002. Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. Am. J. Surg. 183, 445–456.

Braun, S., auf dem Keller, U., Steiling, H., Werner, S., 2004. Fibroblast growth factors in epithelial repair and cytoprotection. Philos. Trans. R. Soc. London, Ser. B: Biol. Sci. 359, 753–757.

Breitkreutz, D., Koxholt, I., Thiemann, K., Nischt, R., 2013. Skin basement membrane: the foundation of epidermal integrity—BM functions and diverse roles of bridging molecules nidogen and perlecan. Biomed. Res. Int. 2013, 179784.

Breitkreutz, D., Mirancea, N., Schmidt, C., Beck, R., Werner, U., Stark, H.J., Gerl, M., Fusenig, N.E., 2004. Inhibition of basement membrane formation by a nidogen-binding laminin gamma1-chain fragment in human skin-organotypic cocultures. J. Cell Sci. 117, 2611–2622.

Breitkreutz, D., Stark, H.J., Mirancea, N., Tomakidi, P., Steinbauer, H., Fusenig, N.E., 1997. Integrin and basement membrane normalization in mouse grafts of human keratinocytes–implications for epidermal homeostasis. Differentiation 61, 195–209, research in biological diversity.

Brem, H., Balledux, J., Bloom, T., Kerstein, M.D., Hollier, L., 2000. Healing of diabetic foot ulcers and pressure ulcers with human skin equivalent: a new paradigm in wound healing. Arch. Surg. 135, 627–634.

Brincat, M.P., 2000. Hormone replacement therapy and the skin. Maturitas 35, 107–117.

Bronneberg, D., Bouten, C.V., Oomens, C.W., van Kemenade, P.M., Baaijens, F.P., 2006. An in vitro model system to study the damaging effects of prolonged mechanical loading of the epidermis. Ann. Biomed. Eng. 34, 506–514.

Bullard, K.M., Longaker, M.T., Lorenz, H.P., 2003. Fetal wound healing: current biology. World J. Surg. 27, 54–61.

Butler, P.D., Ly, D.P., Longaker, M.T., Yang, G.P., 2008. Use of organotypic coculture to study keloid biology. Am. J. Surg. 195, 144–148.

Caplan, A.I., 2007. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J. Cell. Physiol. 213, 341–347.

Cario-Andre, M., Pain, C., Gauthier, Y., Casoli, V., Taieb, A., 2006. In vivo and in vitro evidence of dermal fibroblasts influence on human epidermal pigmentation. Pigment Cell Res. 19, 434–442.

Cario-Andre, M., Pain, C., Gauthier, Y., Taieb, A., 2007. The melanocytorrhagic hypothesis of vitiligo tested on pigmented, stressed, reconstructed epidermis. Pigment Cell Res. 20, 385–393.

Carter, R., Jain, K., Sykes, V., Lanning, D., 2009. Differential expression of procollagen genes between mid- and late-gestational fetal fibroblasts. J. Surg. Res. 156, 90–94.
Castor, C.W., Prince, R.K., Dorstewitz, E.L., 1962. Characteristics of human "fibroblasts" cultivated in vitro from different anatomical sites. Lab. Invest. 11, 703–713, a journal of technical methods and pathology.
Chan, R.K., Zamora, D.O., Wrice, N.L., Baer, D.G., Renz, E.M., Christy, R.J., Natesan, S., 2012. Development of a vascularized skin construct using adipose-derived stem cells from debrided burned skin. Stem Cells Int. 2012, 841203.
Chang, H.Y., Chi, J.T., Dudoit, S., Bondre, C., van de Rijn, M., Botstein, D., Brown, P.O., 2002. Diversity, topographic differentiation, and positional memory in human fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 99, 12877–12882.
Chavez-Munoz, C., Nguyen, K.T., Xu, W., Hong, S.J., Mustoe, T.A., Galiano, R.D., 2013. Transdifferentiation of adipose-derived stem cells into keratinocyte-like cells: engineering a stratified epidermis. PLoS ONE 8, e80587.
Chen, C.S., Lavker, R.M., Rodeck, U., Risse, B., Jensen, P.J., 1995. Use of a serum-free epidermal culture model to show deleterious effects of epidermal growth factor on morphogenesis and differentiation. J. Invest. Dermatol. 104, 107–112.
Cheng, B., Liu, H.W., Fu, X.B., Sheng, Z.Y., Li, J.F., 2008. Coexistence and upregulation of three types of opioid receptors, mu, delta and kappa, in human hypertrophic scars. Br. J. Dermatol. 158, 713–720.
Chinnathambi, S., Tomanek-Chalkley, A., Ludwig, N., King, E., DeWaard, R., Johnson, G., Wertz, P.W., Bickenbach, J.R., 2003. Recapitulation of oral mucosal tissues in long-term organotypic culture. Anat Rec Part A: Discoveries Mol Cell Evol Biol. 270, 162–174.
Chipev, C.C., Simman, R., Hatch, G., Katz, A.E., Siegel, D.M., Simon, M., 2000. Myofibroblast phenotype and apoptosis in keloid and palmar fibroblasts in vitro. Cell Death Differ. 7, 166–176.
Chipev, C.C., Simon, M., 2002. Phenotypic differences between dermal fibroblasts from different body sites determine their responses to tension and TGFbeta1. BMC Dermatol. 2, 13.
Chiu, H.C., Chang, C.H., Chen, J.S., Jee, S.H., 1996. Human hair follicle dermal papilla cell, dermal sheath cell and interstitial dermal fibroblast characteristics. J. Formos. Med. Assoc. 95, 667–674.
Chiu, L.L., Sun, C.H., Yeh, A.T., Torkian, B., Karamzadeh, A., Tromberg, B., Wong, B.J., 2005. Photodynamic therapy on keloid fibroblasts in tissue-engineered keratinocyte-fibroblast co-culture. Lasers Surg. Med. 37, 231–244.
Cho, H.J., Bae, I.H., Chung, H.J., Kim, D.S., Kwon, S.B., Cho, Y.J., Youn, S.W., Park, K.C., 2004. Effects of hair follicle dermal sheath cells in the reconstruction of skin equivalents. J. Dermatol. Sci. 35, 74–77.
Choi, Y., Fuchs, E., 1990. TGF-beta and retinoic acid: regulators of growth and modifiers of differentiation in human epidermal cells. Cell Regul. 1, 791–809.
Chuong, C.M., Cotsarelis, G., Stenn, K., 2007. Defining hair follicles in the age of stem cell bioengineering. J. Invest. Dermatol. 127, 2098–2100.
Colwell, A.S., Beanes, S.R., Soo, C., Dang, C., Ting, K., Longaker, M.T., Atkinson, J.B., Lorenz, H.P., 2005. Increased angiogenesis and expression of vascular endothelial growth factor during scarless repair. Plast. Reconstr. Surg. 115, 204–212.
Commandeur, S., van Drongelen, V., de Gruijl, F.R., El Ghalbzouri, A., 2012. Epidermal growth factor receptor activation and inhibition in 3D in vitro models of normal skin and human cutaneous squamous cell carcinoma. Cancer Sci. 103, 2120–2126.
Contard, P., Bartel, R.L., Jacobs II, L., Perlish, J.S., MacDonald II, E.D., Handler, L., Cone, D., Fleischmajer, R., 1993. Culturing keratinocytes and fibroblasts in a three-dimensional mesh results in epidermal differentiation and formation of a basal lamina-anchoring zone. J. Invest. Dermatol. 100, 35–39.
Cormack, D.H., 1987. The Integumentary System, Ham's Histology, ninth ed. J.B. Lippincott Company, Philadelphia, PA, pp. 450–474.
Costea, D.E., Dimba, A.O., Loro, L.L., Vintermyr, O.K., Johannessen, A.C., 2005. The phenotype of in vitro reconstituted normal human oral epithelium is essentially determined by culture medium. J. Oral. Pathol. Med. 34, 247–252, official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology.
Costea, D.E., Loro, L.L., Dimba, E.A., Vintermyr, O.K., Johannessen, A.C., 2003. Crucial effects of fibroblasts and keratinocyte growth factor on morphogenesis of reconstituted human oral epithelium. J. Invest. Dermatol. 121, 1479–1486.
Couchman, J.R., 1986. Rat hair follicle dermal papillae have an extracellular matrix containing basement membrane components. J. Invest. Dermatol. 87, 762–767.
Crowe, D.L., Hu, L., Gudas, L.J., Rheinwald, J.G., 1991. Variable expression of retinoic acid receptor (RAR beta) mRNA in human oral and epidermal keratinocytes; relation to keratin 19 expression and keratinization potential. Differentiation 48, 199–208, research in biological diversity.
Dang, C.M., Beanes, S.R., Lee, H., Zhang, X., Soo, C., Ting, K., 2003a. Scarless fetal wounds are associated with an increased matrix metalloproteinase-to-tissue-derived inhibitor of metalloproteinase ratio. Plast. Reconstr. Surg. 111, 2273–2285.
Dang, C.M., Beanes, S.R., Soo, C., Ting, K., Benhaim, P., Hedrick, M.H., Lorenz, H.P., 2003b. Decreased expression of fibroblast and keratinocyte growth factor isoforms and receptors during scarless repair. Plast. Reconstr. Surg. 111, 1969–1979.
de Almeida Jr., H., Zigrino, P., Muller, F., Krieg, T., Korge, B., Mauch, C., 2005. Human scalp dermal papilla and fibrous sheath cells have a different expression profile of matrix metalloproteinases in vitro when compared to scalp dermal fibroblasts. Arch. Dermatol. Res. 297, 121–126.
Depner, S., Lederle, W., Gutschalk, C., Linde, N., Zajonz, A., Mueller, M.M., 2014. Cell type specific interleukin-6 induced responses in tumor keratinocytes and stromal fibroblasts are essential for invasive growth. Int. J. Cancer 135, 551–562, Journal international du cancer.
Dhouailly, D., Rogers, G.E., Sengel, P., 1978. The specification of feather and scale protein synthesis in epidermal-dermal recombinations. Dev. Biol. 65, 58–68.
Dongari-Bagtzoglou, A., Kashleva, H., 2006. Development of a highly reproducible three-dimensional organotypic model of the oral mucosa. Nat. Protoc. 1, 2012–2018.
Driskell, R.R., Giangreco, A., Jensen, K.B., Mulder, K.W., Watt, F.M., 2009. Sox2-positive dermal papilla cells specify hair follicle type in mammalian epidermis. Development 136, 2815–2823.
Driskell, R.R., Lichtenberger, B.M., Hoste, E., Kretzschmar, K., Simons, B.D., Charalambous, M., Ferron, S.R., Herault, Y., Pavlovic, G., Ferguson-Smith, A.C., Watt, F.M., 2013. Distinct fibroblast lineages determine dermal architecture in skin development and repair. Nature 504, 277–281.
Duval, C., Regnier, M., Schmidt, R., 2001. Distinct melanogenic response of human melanocytes in mono-culture, in co-culture with keratinocytes and in reconstructed epidermis, to UV exposure. Pigment Cell Res. 14, 348–355.
Eaglstein, W.H., Alvarez, O.M., Auletta, M., Leffel, D., Rogers, G.S., Zitelli, J.A., Norris, J.E., Thomas, I., Irondo, M., Fewkes, J., Hardin-Young, J., Duff, R.G., Sabolinski, M.L., 1999. Acute excisional wounds treated with a tissue-engineered skin (Apligraf). Dermatol. Surg. 25, 195–201, official publication for American Society for Dermatologic Surgery [et al.].
Ebisawa, K., Kato, R., Okada, M., Sugimura, T., Latif, M.A., Hori, Y., Narita, Y., Ueda, M., Honda, H., Kagami, H., 2011. Gingival and dermal fibroblasts: their similarities and differences revealed from gene expression. J. Biosci. Bioeng. 111, 255–258.
Egles, C., Shamis, Y., Mauney, J.R., Volloch, V., Kaplan, D.L., Garlick, J.A., 2008. Denatured collagen modulates the phenotype of normal and wounded human skin equivalents. J. Invest. Dermatol. 128, 1830–1837.
Ehrlich, H.P., Desmouliere, A., Diegelmann, R.F., Cohen, I.K., Compton, C.C., Garner, W.L., Kapanci, Y., Gabbiani, G., 1994. Morphological and immunochemical differences between keloid and hypertrophic scar. Am. J. Pathol. 145, 105–113.
El Ghalbzouri, A., Gibbs, S., Lamme, E., Van Blitterswijk, C.A., Ponec, M., 2002a. Effect of fibroblasts on epidermal regeneration. Br. J. Dermatol. 147, 230–243.
El Ghalbzouri, A., Hensbergen, P., Gibbs, S., Kempenaar, J., van der Schors, R., Ponec, M., 2004. Fibroblasts facilitate re-epithelialization in wounded human skin equivalents. Lab. Invest. 84, 102–112, a journal of technical methods and pathology.
El Ghalbzouri, A., Jonkman, M.F., Dijkman, R., Ponec, M., 2005. Basement membrane reconstruction in human skin equivalents is regulated by fibroblasts and/or exogenously activated keratinocytes. J. Invest. Dermatol. 124, 79–86.
El Ghalbzouri, A., Lamme, E., Ponec, M., 2002b. Crucial role of fibroblasts in regulating epidermal morphogenesis. Cell Tissue Res. 310, 189–199.
Eming, S.A., Krieg, T., Davidson, J.M., 2007. Inflammation in wound repair: molecular and cellular mechanisms. J. Invest. Dermatol. 127, 514–525.
Enoch, S., Peake, M.A., Wall, I., Davies, L., Farrier, J., Giles, P., Kipling, D., Price, P., Moseley, R., Thomas, D., Stephens, P., 2010. 'Young' oral fibroblasts are geno/phenotypically distinct. J. Dent. Res. 89, 1407–1413.
Enoch, S., Wall, I., Peake, M., Davies, L., Farrier, J., Giles, P., Baird, D., Kipling, D., Price, P., Moseley, R., Thomas, D., Stephens, P., 2009. Increased oral fibroblast lifespan is telomerase-independent. J. Dent. Res. 88, 916–921.
Eslami, A., Gallant-Behm, C.L., Hart, D.A., Wiebe, C., Honardoust, D., Gardner, H., Hakkinen, L., Larjava, H.S., 2009. Expression of integrin alphavbeta6 and TGF-beta in scarless vs scar-forming wound healing. J. Histochem. Cytochem. 57, 543–557, official journal of the Histochemistry Society.
Estes, J.M., Adzick, N.S., Harrison, M.R., Longaker, M.T., Stern, R., 1993. Hyaluronate metabolism undergoes an ontogenic transition during fetal development: implications for scar-free wound healing. J. Pediatr. Surg. 28, 1227–1231.
Estrem, S.A., Domayer, M., Bardach, J., Cram, A.E., 1987. Implantation of human keloid into athymic mice. Laryngoscope 97, 1214–1218.
Eves, P., Layton, C., Hedley, S., Dawson, R.A., Wagner, M., Morandini, R., Ghanem, G., Mac Neil, S., 2000. Characterization of an in vitro model of human melanoma invasion based on reconstructed human skin. Br. J. Dermatol. 142, 210–222.
Facy, V., Flouret, V., Regnier, M., Schmidt, R., 2004. Langerhans cells integrated into human reconstructed epidermis respond to known sensitizers and ultraviolet exposure. J. Invest. Dermatol. 122, 552–553.
Facy, V., Flouret, V., Regnier, M., Schmidt, R., 2005. Reactivity of Langerhans cells in human reconstructed epidermis to known allergens and UV radiation. Toxicol. In Vitro 19, 787–795.
Falanga, V., Margolis, D., Alvarez, O., Auletta, M., Maggiacomo, F., Altman, M., Jensen, J., Sabolinski, M., Hardin-Young, J., 1998. Rapid healing of venous ulcers and lack of clinical rejection with an allogeneic cultured human skin equivalent. Human Skin Equivalent Investigators Group. Arch. Dermatol. 134, 293–300.
Fernandes, K.J., McKenzie, I.A., Mill, P., Smith, K.M., Akhavan, M., Barnabe-Heider, F., Biernaskie, J., Junek, A., Kobayashi, N.R., Toma, J.G., Kaplan, D.R., Labosky, P.A., Rafuse, V., Hui, C.C., Miller, F.D., 2004. A dermal niche for multipotent adult skin-derived precursor cells. Nat. Cell Biol. 6, 1082–1093.
Fleischmajer, R., Schechter, A., Bruns, M., Perlish, J.S., Macdonald, E.D., Pan, T.C., Timpl, R., Chu, M.L., 1995. Skin fibroblasts are the only source of nidogen during early basal lamina formation in vitro. J. Invest. Dermatol. 105, 597–601.
Fleischmajer, R., Utani, A., MacDonald, E.D., Perlish, J.S., Pan, T.C., Chu, M.L., Nomizu, M., Ninomiya, Y., Yamada, Y., 1998. Initiation of skin basement membrane formation at the epidermo-dermal interface involves assembly of laminins through binding to cell membrane receptors. J. Cell Sci. 111 (Pt 14), 1929–1940.

Florin, L., Maas-Szabowski, N., Werner, S., Szabowski, A., Angel, P., 2005. Increased keratinocyte proliferation by JUN-dependent expression of PTN and SDF-1 in fibroblasts. J. Cell Sci. 118, 1981–1989.
Fries, K.M., Blieden, T., Looney, R.J., Sempowski, G.D., Silvera, M.R., Willis, R.A., Phipps, R.P., 1994. Evidence of fibroblast heterogeneity and the role of fibroblast subpopulations in fibrosis. Clin. Immunol. Immunopathol. 72, 283–292.
Fu, X., Toh, W.S., Liu, H., Lu, K., Li, M., Hande, M.P., Cao, T., 2010. Autologous feeder cells from embryoid body outgrowth support the long-term growth of human embryonic stem cells more effectively than those from direct differentiation. Tissue Eng. C: Methods 16, 719–733.
Fuchs, E., Green, H., 1981. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. Cell 25, 617–625.
Fujiwara, M., Muragaki, Y., Ooshima, A., 2005. Keloid-derived fibroblasts show increased secretion of factors involved in collagen turnover and depend on matrix metalloproteinase for migration. Br. J. Dermatol. 153, 295–300.
Funayama, E., Chodon, T., Oyama, A., Sugihara, T., 2003. Keratinocytes promote proliferation and inhibit apoptosis of the underlying fibroblasts: an important role in the pathogenesis of keloid. J. Invest. Dermatol. 121, 1326–1331.
Gangatirkar, P., Paquet-Fifield, S., Li, A., Rossi, R., Kaur, P., 2007. Establishment of 3D organotypic cultures using human neonatal epidermal cells. Nat. Protoc. 2, 178–186.
Garzon, I., Miyake, J., Gonzalez-Andrades, M., Carmona, R., Carda, C., Sanchez-Quevedo Mdel, C., Campos, A., Alaminos, M., 2013. Wharton's jelly stem cells: a novel cell source for oral mucosa and skin epithelia regeneration. Stem Cells Transl. Med. 2, 625–632.
Geer, D.J., Swartz, D.D., Andreadis, S.T., 2002. Fibrin promotes migration in a three-dimensional in vitro model of wound regeneration. Tissue Eng. 8, 787–798.
Gharzi, A., Reynolds, A.J., Jahoda, C.A., 2003. Plasticity of hair follicle dermal cells in wound healing and induction. Exp. Dermatol. 12, 126–136.
Gilchrest, B.A., 1996. A review of skin ageing and its medical therapy. Br. J. Dermatol. 135, 867–875.
Giltaire, S., Herphelin, F., Frankart, A., Herin, M., Stoppie, P., Poumay, Y., 2009. The CYP26 inhibitor R115866 potentiates the effects of all-trans retinoic acid on cultured human epidermal keratinocytes. Br. J. Dermatol. 160, 505–513.
Gnedeva, K., Vorotelyak, E., Cimadamore, F., Cattarossi, G., Giusto, E., Terskikh, V.V., Terskikh, A.V., 2015. Derivation of hair-inducing cell from human pluripotent stem cells. PLoS ONE 10, e0116892.
Godin, B., Touitou, E., 2007. Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. Adv. Drug Deliv. Rev. 59, 1152–1161.
Gottlieb, S., Hayes, E., Gilleaudeau, P., Cardinale, I., Gottlieb, A.B., Krueger, J.G., 1996. Cellular actions of etretinate in psoriasis: enhanced epidermal differentiation and reduced cell-mediated inflammation are unexpected outcomes. J. Cutan. Pathol. 23, 404–418.
Grando, S.A., Pittelkow, M.R., Schallreuter, K.U., 2006. Adrenergic and cholinergic control in the biology of epidermis: physiological and clinical significance. J. Invest. Dermatol. 126, 1948–1965.
Grassel, S., Unsold, C., Schacke, H., Bruckner-Tuderman, L., Bruckner, P., 1999. Collagen XVI is expressed by human dermal fibroblasts and keratinocytes and is associated with the microfibrillar apparatus in the upper papillary dermis. Matrix Biol. 18, 309–317.
Griffiths, M., Ojeh, N., Livingstone, R., Price, R., Navsaria, H., 2004. Survival of Apligraf in acute human wounds. Tissue Eng. 10, 1180–1195.
Gschwandtner, M., Mildner, M., Mlitz, V., Gruber, F., Eckhart, L., Werfel, T., Gutzmer, R., Elias, P.M., Tschachler, E., 2013. Histamine suppresses epidermal keratinocyte differentiation and impairs skin barrier function in a human skin model. Allergy 68, 37–47.
Guenou, H., Nissan, X., Larcher, F., Feteira, J., Lemaitre, G., Saidani, M., Del Rio, M., Barrault, C.C., Bernard, F.X., Peschanski, M., Baldeschi, C., Waksman, G., 2009. Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. Lancet 374, 1745–1753.
Gysler, A., Lange, K., Korting, H.C., Schafer-Korting, M., 1997. Prednicarbate biotransformation in human foreskin keratinocytes and fibroblasts. Pharm. Res. 14, 793–797.
Hallock, G.G., Merkel, J.R., Rice, D.C., DiPaolo, B.R., 1993. The ontogenetic transition of collagen deposition in rat skin. Ann. Plast. Surg. 30, 239–243.
Hallock, G.G., Rice, D.C., Merkel, J.R., DiPaolo, B.R., 1988. Analysis of collagen content in the fetal wound. Ann. Plast. Surg. 21, 310–315.
Han, Y., Chai, J., Sun, T., Li, D., Tao, R., 2011. Differentiation of human umbilical cord mesenchymal stem cells into dermal fibroblasts in vitro. Biochem. Biophys. Res. Commun. 413, 561–565.
Hana, A., Booken, D., Henrich, C., Gratchev, A., Maas-Szabowski, N., Goerdt, S., Kurzen, H., 2007. Functional significance of non-neuronal acetylcholine in skin epithelia. Life Sci. 80, 2214–2220.
Harper, R.A., Grove, G., 1979. Human skin fibroblasts derived from papillary and reticular dermis: differences in growth potential in vitro. Science (New York, NY) 204, 526–527.
Harrison, C.A., Heaton, M.J., Layton, C.M., Mac Neil, S., 2006. Use of an in vitro model of tissue-engineered human skin to study keratinocyte attachment and migration in the process of reepithelialization. Wound Repair Regen. 14, 203–209.
Hebda, P.A., Dohar, J.E., 1999. Transplanted fetal fibroblasts: survival and distribution over time in normal adult dermis compared with autogenic, allogenic, and xenogenic adult fibroblasts. Otolaryngol. Head Neck Surg. 121, 245–251, official journal of American Academy of Otolaryngology-Head and Neck Surgery.
Heise, R., Mey, J., Neis, M.M., Marquardt, Y., Joussen, S., Ott, H., Wiederholt, T., Kurschat, P., Megahed, M., Bickers, D.R., Merk, H.F., Baron, J.M., 2006. Skin retinoid concentrations are modulated by CYP26AI expression restricted to basal keratinocytes in normal human skin and differentiated 3D skin models. J. Invest. Dermatol. 126, 2473–2480.
Herskind, C., Bentzen, S.M., Overgaard, J., Overgaard, M., Bamberg, M., Rodemann, H.P., 1998. Differentiation state of skin fibroblast cultures versus risk of subcutaneous fibrosis after radiotherapy. Radiother. Oncol. 47, 263–269, journal of the European Society for Therapeutic Radiology and Oncology.
Hewitt, K.J., Shamis, Y., Carlson, M.W., Aberdam, E., Aberdam, D., Garlick, J.A., 2009. Three-dimensional epithelial tissues generated from human embryonic stem cells. Tissue Eng., A 15, 3417–3426.
Hibberts, N.A., Howell, A.E., Randall, V.A., 1998. Balding hair follicle dermal papilla cells contain higher levels of androgen receptors than those from non-balding scalp. J. Endocrinol. 156, 59–65.
Hibberts, N.A., Messenger, A.G., Randall, V.A., 1996. Dermal papilla cells derived from beard hair follicles secrete more stem cell factor (SCF) in culture than scalp cells or dermal fibroblasts. Biochem. Biophys. Res. Commun. 222, 401–405.
Higashiyama, R., Nakao, S., Shibusawa, Y., Ishikawa, O., Moro, T., Mikami, K., Fukumitsu, H., Ueda, Y., Minakawa, K., Tabata, Y., Bou-Gharios, G., Inagaki, Y., 2011. Differential contribution of dermal resident and bone marrow-derived cells to collagen production during wound healing and fibrogenesis in mice. J. Invest. Dermatol. 131, 529–536.
Hojo, M., Inokuchi, S., Kidokoro, M., Fukuyama, N., Tanaka, E., Tsuji, C., Miyasaka, M., Tanino, R., Nakazawa, H., 2003. Induction of vascular endothelial growth factor by fibrin as a dermal substrate for cultured skin substitute. Plast. Reconstr. Surg. 111, 1638–1645.
Honardoust, D., Ding, J., Varkey, M., Shankowsky, H.A., Tredget, E.E., 2012a. Deep dermal fibroblasts refractory to migration and decorin-induced apoptosis contribute to hypertrophic scarring. J. Burn Care Res. 33, 668–677, official publication of the American Burn Association.
Honardoust, D., Varkey, M., Hori, K., Ding, J., Shankowsky, H.A., Tredget, E.E., 2011. Small leucine-rich proteoglycans, decorin and fibromodulin, are reduced in postburn hypertrophic scar. Wound Repair Regen. 19, 368–378.
Honardoust, D., Varkey, M., Marcoux, Y., Shankowsky, H.A., Tredget, E.E., 2012b. Reduced decorin, fibromodulin, and transforming growth factor-beta3 in deep dermis leads to hypertrophic scarring. J. Burn Care Res. 33, 218–227, official publication of the American Burn Association.
Horne, K.A., Jahoda, C.A., 1992. Restoration of hair growth by surgical implantation of follicular dermal sheath. Development 116, 563–571.
Houzelstein, D., Cheraud, Y., Auda-Boucher, G., Fontaine-Perus, J., Robert, B., 2000. The expression of the homeobox gene Msx1 reveals two populations of dermal progenitor cells originating from the somites. Development 127, 2155–2164.
Hu, S., Kirsner, R.S., Falanga, V., Phillips, T., Eaglstein, W.H., 2006. Evaluation of Apligraf persistence and basement membrane restoration in donor site wounds: a pilot study. Wound Repair Regen. 14, 427–433.
Ihara, S., Motobayashi, Y., Nagao, E., Kistler, A., 1990. Ontogenetic transition of wound healing pattern in rat skin occurring at the fetal stage. Development 110, 671–680.
Ishii, M., Koike, C., Igarashi, A., Yamanaka, K., Pan, H., Higashi, Y., Kawaguchi, H., Sugiyama, M., Kamata, N., Iwata, T., Matsubara, T., Nakamura, K., Kurihara, H., Tsuji, K., Kato, Y., 2005. Molecular markers distinguish bone marrow mesenchymal stem cells from fibroblasts. Biochem. Biophys. Res. Commun. 332, 297–303.
Isseroff, R.R., Chun, K.T., Rosenberg, R.M., 1989. Triiodothyronine alters the cornification of cultured human keratinocytes. Br. J. Dermatol. 120, 503–510.
Itami, S., Kurata, S., Sonoda, T., Takayasu, S., 1995. Interaction between dermal papilla cells and follicular epithelial cells in vitro: effect of androgen. Br. J. Dermatol. 132, 527–532.
Itoh, M., Umegaki-Arao, N., Guo, Z., Liu, L., Higgins, C.A., Christiano, A.M., 2013. Generation of 3D skin equivalents fully reconstituted from human induced pluripotent stem cells (iPSCs). PLoS ONE 8, e77673.
Jahoda, C.A., 1992. Induction of follicle formation and hair growth by vibrissa dermal papillae implanted into rat ear wounds: vibrissa-type fibres are specified. Development 115, 1103–1109.
Jahoda, C.A., 2003. Cell movement in the hair follicle dermis—more than a two-way street? J. Invest. Dermatol. 121, ix–xi.
Jahoda, C.A., Horne, K.A., Oliver, R.F., 1984. Induction of hair growth by implantation of cultured dermal papilla cells. Nature 311, 560–562.
Jahoda, C.A., Reynolds, A.J., 2001. Hair follicle dermal sheath cells: unsung participants in wound healing. Lancet 358, 1445–1448.
Jahoda, C.A., Reynolds, A.J., Chaponnier, C., Forester, J.C., Gabbiani, G., 1991. Smooth muscle alpha-actin is a marker for hair follicle dermis in vivo and in vitro. J. Cell Sci. 99 (Pt 3), 627–636.
Jahoda, C.A., Whitehouse, J., Reynolds, A.J., Hole, N., 2003. Hair follicle dermal cells differentiate into adipogenic and osteogenic lineages. Exp. Dermatol. 12, 849–859.
Janson, D., Saintigny, G., Mahe, C., El Ghalbzouri, A., 2013. Papillary fibroblasts differentiate into reticular fibroblasts after prolonged in vitro culture. Exp. Dermatol. 22, 48–53.
Janson, D.G., Saintigny, G., van Adrichem, A., Mahe, C., El Ghalbzouri, A., 2012. Different gene expression patterns in human papillary and reticular fibroblasts. J. Invest. Dermatol. 132, 2565–2572.

Jean, J., Lapointe, M., Soucy, J., Pouliot, R., 2009. Development of an in vitro psoriatic skin model by tissue engineering. J. Dermatol. Sci. 53, 19–25.
Jean, J., Soucy, J., Pouliot, R., 2011. Effects of retinoic acid on keratinocyte proliferation and differentiation in a psoriatic skin model. Tissue Eng., A 17, 1859–1868.
Jones, J.E., Nelson, E.A., 2005. Skin grafting for venous leg ulcers. In: The Cochrane Database of Systematic Reviews. John Wiley & Sons. Ltd, CD001737.
Jumper, N., Paus, R., Bayat, A., 2015. Functional histopathology of keloid disease. Histol. Histopathol. 30 (9), 1033–1057.
Kamel, R.A., Ong, J.F., Eriksson, E., Junker, J.P., Caterson, E.J., 2013. Tissue engineering of skin. J. Am. Coll. Surg. 217, 533–555.
Kanda, N., Morimoto, N., Takemoto, S., Ayvazyan, A.A., Kawai, K., Sakamoto, Y., Taira, T., Suzuki, S., 2012. Efficacy of novel collagen/gelatin scaffold with sustained release of basic fibroblast growth factor for dermis-like tissue regeneration. Ann. Plast. Surg. 69, 569–574.
Kaplan, M.M., Pan, C.Y., Gordon, P.R., Lee, J.K., Gilchrest, B.A., 1988. Human epidermal keratinocytes in culture convert thyroxine to 3,5,3′-triiodothyronine by type II iodothyronine deiodination: a novel endocrine function of the skin. J. Clin. Endocrinol. Metab. 66, 815–822.
Kassner, A., Hansen, U., Miosge, N., Reinhardt, D.P., Aigner, T., Bruckner-Tuderman, L., Bruckner, P., Grassel, S., 2003. Discrete integration of collagen XVI into tissue-specific collagen fibrils or beaded microfibrils. Matrix Biol. 22, 131–143.
Kataoka, K., Medina, R.J., Kageyama, T., Miyazaki, M., Yoshino, T., Makino, T., Huh, N.H., 2003. Participation of adult mouse bone marrow cells in reconstitution of skin. Am. J. Pathol. 163, 1227–1231.
Kautsky, M.B., Fleckman, P., Dale, B.A., 1995. Retinoic acid regulates oral epithelial differentiation by two mechanisms. J. Invest. Dermatol. 104, 546–553.
Kawai, K., Suzuki, S., Tabata, Y., Ikada, Y., Nishimura, Y., 2000. Accelerated tissue regeneration through incorporation of basic fibroblast growth factor-impregnated gelatin microspheres into artificial dermis. Biomaterials 21, 489–499.
Kennedy, C.I., Diegelmann, R.F., Haynes, J.H., Yager, D.R., 2000. Proinflammatory cytokines differentially regulate hyaluronan synthase isoforms in fetal and adult fibroblasts. J. Pediatr. Surg. 35, 874–879.
Kidwai, F.K., Jokhun, D.S., Movahednia, M.M., Yeo, J.F., Tan, K.S., Cao, T., 2013. Human embryonic stem cells derived keratinocyte as an in vitro research model for the study of immune response. J. Oral Pathol. Med. 42, 627–634, official publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology.
Kim, S.R., Cha, S.Y., Kim, M.K., Kim, J.C., Sung, Y.K., 2006. Induction of versican by ascorbic acid 2-phosphate in dermal papilla cells. J. Dermatol. Sci. 43, 60–62.
Kishimoto, J., Ehama, R., Wu, L., Jiang, S., Jiang, N., Burgeson, R.E., 1999. Selective activation of the versican promoter by epithelial- mesenchymal interactions during hair follicle development. Proc. Natl. Acad. Sci. U.S.A. 96, 7336–7341.
Kollar, E.J., 1986. Tissue interactions in development of teeth and related ectodermal derivatives. Dev. Biol. (N.Y. 1985) 4, 297–313.
Kolokol'chikova, E.G., Budkevich, L.I., Bobrovnikov, A.E., Badikova, A.K., Tumanov, V.P., 2001. Morphological changes in burn wounds after transplantation of allogenic fibroblasts. Bull. Exp. Biol. Med. 131, 89–93.
König, A., Bruckner-Tuderman, L., 1991. Epithelial-mesenchymal interactions enhance expression of collagen VII in vitro. J. Invest. Dermatol. 96, 803–808.
König, A., Bruckner-Tuderman, L., 1992. Transforming growth factor-beta stimulates collagen VII expression by cutaneous cells in vitro. J. Cell Biol. 117, 679–685.
König, A., Bruckner-Tuderman, L., 1994. Transforming growth factor-beta promotes deposition of collagen VII in a modified organotypic skin model. Lab. Invest. 70, 203–209, a journal of technical methods and pathology.
Kopan, R., Traska, G., Fuchs, E., 1987. Retinoids as important regulators of terminal differentiation: examining keratin expression in individual epidermal cells at various stages of keratinization. J. Cell Biol. 105, 427–440.
Kriegebaum, U., Mildenberger, M., Mueller-Richter, U.D., Klammert, U., Kuebler, A.C., Reuther, T., 2012. Tissue engineering of human oral mucosa on different scaffolds: in vitro experiments as a basis for clinical applications. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. 114, S190–S198.
Kuchler, S., Henkes, D., Eckl, K.M., Ackermann, K., Plendl, J., Korting, H.C., Hennies, H.C., Schafer-Korting, M., 2011. Hallmarks of atopic skin mimicked in vitro by means of a skin disease model based on FLG knock-down. Altern. Lab. Anim. 39, 471–480.
Kurzen, H., Henrich, C., Booken, D., Poenitz, N., Gratchev, A., Klemke, C.D., Engstner, M., Goerdt, S., Maas-Szabowski, N., 2006. Functional characterization of the epidermal cholinergic system in vitro. J. Invest. Dermatol. 126, 2458–2472.
Kurzen, H., Wessler, I., Kirkpatrick, C.J., Kawashima, K., Grando, S.A., 2007. The non-neuronal cholinergic system of human skin. Horm. Metab. Res. 39, 125–135.
Lamb, R., Ambler, C.A., 2013. Keratinocytes propagated in serum-free, feeder-free culture conditions fail to form stratified epidermis in a reconstituted skin model. PLoS ONE 8, e52494.
Lamme, E.N., Van Leeuwen, R.T., Brandsma, K., Van Marle, J., Middelkoop, E., 2000. Higher numbers of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar tissue formation. J. Pathol. 190, 595–603.
Lamme, E.N., van Leeuwen, R.T., Mekkes, J.R., Middelkoop, E., 2002. Allogeneic fibroblasts in dermal substitutes induce inflammation and scar formation. Wound Repair Regen. 10, 152–160.
Laplante, A.F., Germain, L., Auger, F.A., Moulin, V., 2001. Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. FASEB J. 15, 2377–2389.
Larson, B.J., Longaker, M.T., Lorenz, H.P., 2010. Scarless fetal wound healing: a basic science review. Plast. Reconstr. Surg. 126, 1172–1180.
Le Lievre, C.S., Le Douarin, N.M., 1975. Mesenchymal derivatives of the neural crest: analysis of chimaeric quail and chick embryos. J. Embryol. Exp. Morph. 34, 125–154.
Lee, C.H., Shah, B., Moioli, E.K., Mao, J.J., 2010. CTGF directs fibroblast differentiation from human mesenchymal stem/stromal cells and defines connective tissue healing in a rodent injury model. J. Clin. Invest. 120, 3340–3349.
Lee, D.Y., Cho, K.H., 2005. The effects of epidermal keratinocytes and dermal fibroblasts on the formation of cutaneous basement membrane in three-dimensional culture systems. Arch. Dermatol. Res. 296, 296–302.
Lee, H.G., Eun, H.C., 1999. Differences between fibroblasts cultured from oral mucosa and normal skin: implication to wound healing. J. Dermatol. Sci. 21, 176–182.
Lee, J.Y., Yang, C.C., Chao, S.C., Wong, T.W., 2004. Histopathological differential diagnosis of keloid and hypertrophic scar. Am. J. Dermatopathol. 26, 379–384.
Lee, W.J., Choi, I.K., Lee, J.H., Kim, Y.O., Yun, C.O., 2013. A novel three-dimensional model system for keloid study: organotypic multicellular scar model. Wound Repair Regen. 21, 155–165.
Li, L., Fukunaga-Kalabis, M., Herlyn, M., 2011. The three-dimensional human skin reconstruct model: a tool to study normal skin and melanoma progression. J. Vis. Exp. (54), http://dx.doi.org/10.3791/2937, pii: 2937.
Li, L., Shukla, S., Lee, A., Garfield, S.H., Maloney, D.J., Ambudkar, S.V., Yuspa, S.H., 2010. The skin cancer chemotherapeutic agent ingenol-3-angelate (PEP005) is a substrate for the epidermal multidrug transporter (ABCB1) and targets tumor vasculature. Cancer Res. 70, 4509–4519.
Lim, I.J., Phan, T.T., Bay, B.H., Qi, R., Huynh, H., Tan, W.T., Lee, S.T., Longaker, M.T., 2002. Fibroblasts cocultured with keloid keratinocytes: normal fibroblasts secrete collagen in a keloidlike manner. Am. J. Phys.: Cell Phys. 283, C212–C222.
Lim, I.J., Phan, T.T., Song, C., Tan, W.T., Longaker, M.T., 2001. Investigation of the influence of keloid-derived keratinocytes on fibroblast growth and proliferation in vitro. Plast. Reconstr. Surg. 107, 797–808.
Liu, Y., Suwa, F., Wang, X., Takemura, A., Fang, Y.R., Li, Y., Zhao, Y., Jin, Y., 2007. Reconstruction of a tissue-engineered skin containing melanocytes. Cell Biol. Int. 31, 985–990.
Longaker, M.T., Adzick, N.S., Hall, J.L., Stair, S.E., Crombleholme, T.M., Duncan, B.W., Bradley, S.M., Harrison, M.R., Stern, R., 1990a. Studies in fetal wound healing, VII. Fetal wound healing may be modulated by hyaluronic acid stimulating activity in amniotic fluid. J. Pediatr. Surg. 25, 430–433.
Longaker, M.T., Chiu, E.S., Adzick, N.S., Stern, M., Harrison, M.R., Stern, R., 1991. Studies in fetal wound healing, V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. Ann. Surg. 213, 292–296.
Longaker, M.T., Chiu, E.S., Harrison, M.R., Crombleholme, T.M., Langer, J.C., Duncan, B.W., Adzick, N.S., Verrier, E.D., Stern, R., 1989. Studies in fetal wound healing. IV. Hyaluronic acid-stimulating activity distinguishes fetal wound fluid from adult wound fluid. Ann. Surg. 210, 667–672.
Longaker, M.T., Whitby, D.J., Adzick, N.S., Crombleholme, T.M., Langer, J.C., Duncan, B.W., Bradley, S.M., Stern, R., Ferguson, M.W., Harrison, M.R., 1990b. Studies in fetal wound healing, VI. Second and early third trimester fetal wounds demonstrate rapid collagen deposition without scar formation. J. Pediatr. Surg. 25, 63–68, discussion 68-69.
Longaker, M.T., Whitby, D.J., Ferguson, M.W., Lorenz, H.P., Harrison, M.R., Adzick, N.S., 1994. Adult skin wounds in the fetal environment heal with scar formation. Ann. Surg. 219, 65–72.
Lorenz, H.P., Lin, R.Y., Longaker, M.T., Whitby, D.J., Adzick, N.S., 1995. The fetal fibroblast: the effector cell of scarless fetal skin repair. Plast. Reconstr. Surg. 96, 1251–1259, discussion 1260-1251.
Lorenz, H.P., Longaker, M.T., Perkocha, L.A., Jennings, R.W., Harrison, M.R., Adzick, N.S., 1992. Scarless wound repair: a human fetal skin model. Development 114, 253–259.
Lorenz, H.P., Whitby, D.J., Longaker, M.T., Adzick, N.S., 1993. Fetal wound healing. The ontogeny of scar formation in the non-human primate. Ann. Surg. 217, 391–396.
Lukandu, O.M., Neppelberg, E., Vintermyr, O.K., Johannessen, A.C., Costea, D.E., 2010. Khat alters the phenotype of in vitro-reconstructed human oral mucosa. J. Dent. Res. 89, 270–275.
Lygoe, K.A., Wall, I., Stephens, P., Lewis, M.P., 2007. Role of vitronectin and fibronectin receptors in oral mucosal and dermal myofibroblast differentiation. Biol. Cell 99, 601–614, under the auspices of the European Cell Biology Organization.
Maas-Szabowski, N., Fusenig, N.E., 1996. Interleukin-1-induced growth factor expression in postmitotic and resting fibroblasts. J. Invest. Dermatol. 107, 849–855.
Maas-Szabowski, N., Shimotoyodome, A., Fusenig, N.E., 1999. Keratinocyte growth regulation in fibroblast cocultures via a double paracrine mechanism. J. Cell Sci. 112 (Pt 12), 1843–1853.
Maas-Szabowski, N., Stark, H.J., Fusenig, N.E., 2000. Keratinocyte growth regulation in defined organotypic cultures through IL-1-induced keratinocyte growth factor expression in resting fibroblasts. J. Invest. Dermatol. 114, 1075–1084.
Maas-Szabowski, N., Szabowski, A., Stark, H.J., Andrecht, S., Kolbus, A., Schorpp-Kistner, M., Angel, P., Fusenig, N.E., 2001. Organotypic cocultures with genetically modified mouse fibroblasts as a tool to dissect molecular

mechanisms regulating keratinocyte growth and differentiation. J. Invest. Dermatol. 116, 816–820.
Machesney, M., Tidman, N., Waseem, A., Kirby, L., Leigh, I., 1998. Activated keratinocytes in the epidermis of hypertrophic scars. Am. J. Pathol. 152, 1133–1141.
MacNeil, S., 2007. Progress and opportunities for tissue-engineered skin. Nature 445, 874–880.
MacNeil, S., Shepherd, J., Smith, L., 2011. Production of tissue-engineered skin and oral mucosa for clinical and experimental use. Methods Mol. Biol. 695, 129–153.
Mah, W., Jiang, G., Olver, D., Cheung, G., Kim, B., Larjava, H., Hakkinen, L., 2014. Human gingival fibroblasts display a non-fibrotic phenotype distinct from skin fibroblasts in three-dimensional cultures. PLoS ONE 9, e90715.
Malacinski, G.M., Bryant, S.V., 1984. Pattern Formation: A Primer in Developmental Biology. Macmillan Pub. Co., New York, NY.
Mancini, M., Saintigny, G., Mahe, C., Annicchiarico-Petruzzelli, M., Melino, G., Candi, E., 2012. MicroRNA-152 and -181a participate in human dermal fibroblasts senescence acting on cell adhesion and remodeling of the extra-cellular matrix. Aging (Albany NY) 4, 843–853.
Marinkovich, M.P., Keene, D.R., Rimberg, C.S., Burgeson, R.E., 1993. Cellular origin of the dermal-epidermal basement membrane. Dev. Dyn. 197, 255–267, an official publication of the American Association of Anatomists.
Marino, D., Luginbuhl, J., Scola, S., Meuli, M., Reichmann, E., 2014. Bioengineering dermo-epidermal skin grafts with blood and lymphatic capillaries. Sci. Transl. Med. 6, 221ra214.
Marrero, B., Heller, R., 2012. The use of an in vitro 3D melanoma model to predict in vivo plasmid transfection using electroporation. Biomaterials 33, 3036–3046.
Marston, W.A., Hanft, J., Norwood, P., Pollak, R., Dermagraft Diabetic Foot Ulcer Study, G., 2003. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. Diabetes Care 26, 1701–1705.
Mast, B.A., Diegelmann, R.F., Krummel, T.M., Cohen, I.K., 1993. Hyaluronic acid modulates proliferation, collagen and protein synthesis of cultured fetal fibroblasts. Matrix 13, 441–446.
McElwee, K.J., Kissling, S., Wenzel, E., Huth, A., Hoffmann, R., 2003. Cultured peribulbar dermal sheath cells can induce hair follicle development and contribute to the dermal sheath and dermal papilla. J. Invest. Dermatol. 121, 1267–1275.
McKeown, S.T., Barnes, J.J., Hyland, P.L., Lundy, F.T., Fray, M.J., Irwin, C.R., 2007. Matrix metalloproteinase-3 differences in oral and skin fibroblasts. J. Dent. Res. 86, 457–462.
McMillan, J.R., Akiyama, M., Shimizu, H., 2003. Epidermal basement membrane zone components: ultrastructural distribution and molecular interactions. J. Dermatol. Sci. 31, 169–177.
Mendoza, J., Sebastian, A., Allan, E., Allan, D., Mandal, P., Alonso-Rasgado, T., Bayat, A., 2012. Differential cytotoxic response in keloid fibroblasts exposed to photodynamic therapy is dependent on photosensitiser precursor, fluence and location of fibroblasts within the lesion. Arch. Dermatol. Res. 304, 549–562.
Meran, S., Thomas, D., Stephens, P., Martin, J., Bowen, T., Phillips, A., Steadman, R., 2007. Involvement of hyaluronan in regulation of fibroblast phenotype. J. Biol. Chem. 282, 25687–25697.
Meran, S., Thomas, D.W., Stephens, P., Enoch, S., Martin, J., Steadman, R., Phillips, A.O., 2008. Hyaluronan facilitates transforming growth factor-beta1-mediated fibroblast proliferation. J. Biol. Chem. 283, 6530–6545.
Merkel, J.R., DiPaolo, B.R., Hallock, G.G., Rice, D.C., 1988. Type I and type III collagen content of healing wounds in fetal and adult rats. Proc. Soc. Exp. Biol. Med.: Soc. Exp. Biol. Med. 187, 493–497.
Messenger, A.G., Senior, H.J., Bleehen, S.S., 1986. The in vitro properties of dermal papilla cell lines established from human hair follicles. Br. J. Dermatol. 114, 425–430.
Metallo, C.M., Azarin, S.M., Moses, L.E., Ji, L., de Pablo, J.J., Palecek, S.P., 2010. Human embryonic stem cell-derived keratinocytes exhibit an epidermal transcription program and undergo epithelial morphogenesis in engineered tissue constructs. Tissue Eng., A 16, 213–223.
Metallo, C.M., Ji, L., de Pablo, J.J., Palecek, S.P., 2008. Retinoic acid and bone morphogenetic protein signaling synergize to efficiently direct epithelial differentiation of human embryonic stem cells. Stem Cells 26, 372–380.
Metcalfe, A.D., Ferguson, M.W., 2007. Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J. R. Soc. Interface 4, 413–437, the Royal Society.
Mildner, M., Jin, J., Eckhart, L., Kezic, S., Gruber, F., Barresi, C., Stremnitzer, C., Buchberger, M., Mlitz, V., Ballaun, C., Sterniczky, B., Fodinger, D., Tschachler, E., 2010. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J. Invest. Dermatol. 130, 2286–2294.
Mills, S.J., Cowin, A.J., Kaur, P., 2013. Pericytes, mesenchymal stem cells and the wound healing process. Cells 2, 621–634.
Mine, S., Fortunel, N.O., Pageon, H., Asselineau, D., 2008. Aging alters functionally human dermal papillary fibroblasts but not reticular fibroblasts: a new view of skin morphogenesis and aging. PLoS ONE 3, e4066.
Miner, J.H., 2008. Laminins and their roles in mammals. Microsc. Res. Tech. 71, 349–356.
Miner, J.H., Yurchenco, P.D., 2004. Laminin functions in tissue morphogenesis. Annu. Rev. Cell Dev. Biol. 20, 255–284.
Moharamzadeh, K., Colley, H., Murdoch, C., Hearnden, V., Chai, W.L., Brook, I.M., Thornhill, M.H., Macneil, S., 2012. Tissue-engineered oral mucosa. J. Dent. Res. 91, 642–650.
Mokkapati, S., Baranowsky, A., Mirancea, N., Smyth, N., Breitkreutz, D., Nischt, R., 2008. Basement membranes in skin are differently affected by lack of nidogen 1 and 2. J. Invest. Dermatol. 128, 2259–2267.
Mokkapati, S., Fleger-Weckmann, A., Bechtel, M., Koch, M., Breitkreutz, D., Mayer, U., Smyth, N., Nischt, R., 2011. Basement membrane deposition of nidogen 1 but not nidogen 2 requires the nidogen binding module of the laminin gamma1 chain. J. Biol. Chem. 286, 1911–1918.
Moon, P.G., Kwack, M.H., Lee, J.E., Cho, Y.E., Park, J.H., Hwang, D., Kim, M.K., Kim, J.C., Sung, Y.K., Baek, M.C., 2013. Proteomic analysis of balding and non-balding mesenchyme-derived dermal papilla cells from androgenetic alopecia patients using on-line two-dimensional reversed phase-reversed phase LC-MS/MS. J. Proteomics 85, 174–191.
Morimoto, N., Saso, Y., Tomihata, K., Taira, T., Takahashi, Y., Ohta, M., Suzuki, S., 2005. Viability and function of autologous and allogeneic fibroblasts seeded in dermal substitutes after implantation. J. Surg. Res. 125, 56–67.
Moulin, V., Garrel, D., Auger, F.A., O'Connor-McCourt, M., Castilloux, G., Germain, L., 1999. What's new in human wound-healing myofibroblasts? Current topics in pathology. Ergebnisse der Pathologie 93, 123–133.
Mueller, M.M., Fusenig, N.E., 2002. Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. Differentiation 70, 486–497, research in biological diversity.
Muffler, S., Stark, H.J., Amoros, M., Falkowska-Hansen, B., Boehnke, K., Buhring, H.J., Marme, A., Bickenbach, J.R., Boukamp, P., 2008. A stable niche supports long-term maintenance of human epidermal stem cells in organotypic cultures. Stem Cells 26, 2506–2515.
Mukhopadhyay, A., Chan, S.Y., Lim, I.J., Phillips, D.J., Phan, T.T., 2007. The role of the activin system in keloid pathogenesis. Am. J. Phys.: Cell Phys. 292, C1331–C1338.
Mukhopadhyay, A., Tan, E.K., Khoo, Y.T., Chan, S.Y., Lim, I.J., Phan, T.T., 2005. Conditioned medium from keloid keratinocyte/keloid fibroblast coculture induces contraction of fibroblast-populated collagen lattices. Br. J. Dermatol. 152, 639–645.
Neumann, C., Bigliardi-Qi, M., Widmann, C., Bigliardi, P.L., 2015. The delta-opioid receptor affects epidermal homeostasis via ERK-dependent inhibition of transcription factor POU2F3. J. Invest. Dermatol. 135 (2), 471–480.
Nguyen, V.T., Chernyavsky, A.I., Arredondo, J., Bercovich, D., Orr-Urtreger, A., Vetter, D.E., Wess, J., Beaudet, A.L., Kitajima, Y., Grando, S.A., 2004. Synergistic control of keratinocyte adhesion through muscarinic and nicotinic acetylcholine receptor subtypes. Exp. Cell Res. 294, 534–549.
Niessen, F.B., Andriessen, M.P., Schalkwijk, J., Visser, L., Timens, W., 2001. Keratinocyte-derived growth factors play a role in the formation of hypertrophic scars. J. Pathol. 194, 207–216.
Nischt, R., Schmidt, C., Mirancea, N., Baranowsky, A., Mokkapati, S., Smyth, N., Woenne, E.C., Stark, H.J., Boukamp, P., Breitkreutz, D., 2007. Lack of nidogen-1 and -2 prevents basement membrane assembly in skin-organotypic coculture. J. Invest. Dermatol. 127, 545–554.
Nolte, S.V., Xu, W., Rennekampff, H.O., Rodemann, H.P., 2008. Diversity of fibroblasts—a review on implications for skin tissue engineering. Cells Tissues Organs 187, 165–176.
Occleston, N.L., O'Kane, S., Goldspink, N., Ferguson, M.W., 2008. New therapeutics for the prevention and reduction of scarring. Drug Discovery Today 13, 973–981.
Occleston, N.L., O'Kane, S., Laverty, H.G., Cooper, M., Fairlamb, D., Mason, T., Bush, J.A., Ferguson, M.W., 2011. Discovery and development of avotermin (recombinant human transforming growth factor beta 3): a new class of prophylactic therapeutic for the improvement of scarring. Wound Repair Regen. 19 (Suppl 1), s38–s48.
Ohtola, J., Myers, J., Akhtar-Zaidi, B., Zuzindlak, D., Sandesara, P., Yeh, K., Mackem, S., Atit, R., 2008. beta-Catenin has sequential roles in the survival and specification of ventral dermis. Development 135, 2321–2329.
Ohyama, M., Zheng, Y., Paus, R., Stenn, K.S., 2010. The mesenchymal component of hair follicle neogenesis: background, methods and molecular characterization. Exp. Dermatol. 19, 89–99.
Okamoto, E., Kitano, Y., 1993. Expression of basement membrane components in skin equivalents—influence of dermal fibroblasts. J. Dermatol. Sci. 5, 81–88.
Okazaki, M., Yoshimura, K., Uchida, G., Harii, K., 2002. Elevated expression of hepatocyte and keratinocyte growth factor in cultured buccal-mucosa-derived fibroblasts compared with normal-skin-derived fibroblasts. J. Dermatol. Sci. 30, 108–115.
Oliver, R.F., 1967. The experimental induction of whisker growth in the hooded rat by implantation of dermal papillae. J. Embryol. Exp. Morph. 18, 43–51.
Oliver, R.F., 1970. The induction of hair follicle formation in the adult hooded rat by vibrissa dermal papillae. J. Embryol. Exp. Morph. 23, 219–236.
Pageon, H., 2010. Reaction of glycation and human skin: the effects on the skin and its components, reconstructed skin as a model. Pathol.–Biol. 58, 226–231.
Pageon, H., Bakala, H., Monnier, V.M., Asselineau, D., 2007. Collagen glycation triggers the formation of aged skin in vitro. Eur. J. Dermatol.: EJD 17, 12–20.
Pageon, H., Zucchi, H., Asselineau, D., 2012. Distinct and complementary roles of papillary and reticular fibroblasts in skin morphogenesis and homeostasis. Eur. J. Dermatol.: EJD 22, 324–332.
Paquet-Fifield, S., Schluter, H., Li, A., Aitken, T., Gangatirkar, P., Blashki, D., Koelmeyer, R., Pouliot, N., Palatsides, M., Ellis, S., Brouard, N., Zannettino, A., Saunders, N., Thompson, N., Li, J., Kaur, P., 2009. A role for pericytes as

microenvironmental regulators of human skin tissue regeneration. J. Clin. Invest. 119, 2795–2806.
Pasonen-Seppanen, S., Suhonen, T.M., Kirjavainen, M., Suihko, E., Urtti, A., Miettinen, M., Hyttinen, M., Tammi, M., Tammi, R., 2001. Vitamin C enhances differentiation of a continuous keratinocyte cell line (REK) into epidermis with normal stratum corneum ultrastructure and functional permeability barrier. Histochem. Cell Biol. 116, 287–297.
Patel, M., Fisher, J.P., 2008. Biomaterial scaffolds in pediatric tissue engineering. Pediatr. Res. 63, 497–501.
Petrova, A., Celli, A., Jacquet, L., Dafou, D., Crumrine, D., Hupe, M., Arno, M., Hobbs, C., Cvoro, A., Karagiannis, P., Devito, L., Sun, R., Adame, L.C., Vaughan, R., McGrath, J.A., Mauro, T.M., Ilic, D., 2014. 3D in vitro model of a functional epidermal permeability barrier from human embryonic stem cells and induced pluripotent stem cells. Stem Cell Rep. 2, 675–689.
Phan, T.T., Lim, I.J., Bay, B.H., Qi, R., Huynh, H.T., Lee, S.T., Longaker, M.T., 2002. Differences in collagen production between normal and keloid-derived fibroblasts in serum-media co-culture with keloid-derived keratinocytes. J. Dermatol. Sci. 29, 26–34.
Phan, T.T., Lim, I.J., Bay, B.H., Qi, R., Longaker, M.T., Lee, S.T., Huynh, H., 2003a. Role of IGF system of mitogens in the induction of fibroblast proliferation by keloid-derived keratinocytes in vitro. Am. J. Phys.: Cell Phys. 284, C860–C869.
Phan, T.T., Sun, L., Bay, B.H., Chan, S.Y., Lee, S.T., 2003b. Dietary compounds inhibit proliferation and contraction of keloid and hypertrophic scar-derived fibroblasts in vitro: therapeutic implication for excessive scarring. J. Trauma 54, 1212–1224.
Phillips, T.J., Manzoor, J., Rojas, A., Isaacs, C., Carson, P., Sabolinski, M., Young, J., Falanga, V., 2002. The longevity of a bilayered skin substitute after application to venous ulcers. Arch. Dermatol. 138, 1079–1081.
Ponec, M., Gibbs, S., Pilgram, G., Boelsma, E., Koerten, H., Bouwstra, J., Mommaas, M., 2001. Barrier function in reconstructed epidermis and its resemblance to native human skin. Skin Pharmacol. Appl. Skin Physiol. 14 (Suppl. 1), 63–71.
Ponec, M., Weerheim, A., Kempenaar, J., Mulder, A., Gooris, G.S., Bouwstra, J., Mommaas, A.M., 1997. The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. J. Invest. Dermatol. 109, 348–355.
Pontiggia, L., Klar, A., Bottcher-Haberzeth, S., Biedermann, T., Meuli, M., Reichmann, E., 2013. Optimizing in vitro culture conditions leads to a significantly shorter production time of human dermo-epidermal skin substitutes. Pediatr. Surg. Int. 29, 249–256.
Pouliot, N., Saunders, N.A., Kaur, P., 2002. Laminin 10/11: an alternative adhesive ligand for epidermal keratinocytes with a functional role in promoting proliferation and migration. Exp. Dermatol. 11, 387–397.
Pouyani, T., Papp, S., Schaffer, L., 2012. Tissue-engineered fetal dermal matrices. In vitro cellular & developmental biology. Animal 48, 493–506.
Price, R.D., Das-Gupta, V., Harris, P.A., Leigh, I.M., Navsaria, H.A., 2004. The role of allogenic fibroblasts in an acute wound healing model. Plast. Reconstr. Surg. 113, 1719–1729.
Pugliese, P.T., 2007. The pathogenesis of cellulite: a new concept. J. Cos. Dermatol. 6, 140–142.
Pujuguet, P., Simian, M., Liaw, J., Timpl, R., Werb, Z., Bissell, M.J., 2000. Nidogen-1 regulates laminin-1-dependent mammary-specific gene expression. J. Cell Sci. 113 (Pt 5), 849–858.
Raap, U., Kapp, A., 2010. Neurotrophins in healthy and diseased skin. G. Ital. Dermatol. Venereol. 145, 205–211.
Radoja, N., Komine, M., Jho, S.H., Blumenberg, M., Tomic-Canic, M., 2000. Novel mechanism of steroid action in skin through glucocorticoid receptor monomers. Mol. Cell Biol. 20, 4328–4339.
Randall, V.A., Hibberts, N.A., Hamada, K., 1996. A comparison of the culture and growth of dermal papilla cells from hair follicles from non-balding and balding (androgenetic alopecia) scalp. Br. J. Dermatol. 134, 437–444.
Randall, V.A., Thornton, M.J., Hamada, K., Messenger, A.G., 1992. Mechanism of androgen action in cultured dermal papilla cells derived from human hair follicles with varying responses to androgens in vivo. J. Invest. Dermatol. 98, 86S–91S.
Regnier, M., Caron, D., Reichert, U., Schaefer, H., 1993. Barrier function of human skin and human reconstructed epidermis. J. Pharm. Sci. 82, 404–407.
Rendl, M., Lewis, L., Fuchs, E., 2005. Molecular dissection of mesenchymal-epithelial interactions in the hair follicle. PLoS Biol. 3, e331.
Reynolds, A.J., Lawrence, C., Cserhalmi-Friedman, P.B., Christiano, A.M., Jahoda, C.A., 1999. Trans-gender induction of hair follicles. Nature 402, 33–34.
Rhett, J.M., Ghatnekar, G.S., Palatinus, J.A., O'Quinn, M., Yost, M.J., Gourdie, R.G., 2008. Novel therapies for scar reduction and regenerative healing of skin wounds. Trends Biotechnol. 26, 173–180.
Rinkevich, Y., Walmsley, G.G., Hu, M.S., Maan, Z.N., Newman, A.M., Drukker, M., Januszyk, M., Krampitz, G.W., Gurtner, G.C., Lorenz, H.P., Weissman, I.L., Longaker, M.T., 2015. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science (New York, N.Y.) 348, aaa2151.
Rinn, J.L., Bondre, C., Gladstone, H.B., Brown, P.O., Chang, H.Y., 2006. Anatomic demarcation by positional variation in fibroblast gene expression programs. PLoS Genet. 2, e119.
Robb, E.C., Waymack, J.P., Warden, G.D., Nathan, P., Alexander, J.W., 1987. A new model for studying the development of human hypertrophic burn scar formation. J. Burn Care Rehab. 8, 371–375.
Rodemann, H.P., Bayreuther, K., Francz, P.I., Dittmann, K., Albiez, M., 1989a. Selective enrichment and biochemical characterization of seven human skin fibroblasts cell types in vitro. Exp. Cell Res. 180, 84–93.
Rodemann, H.P., Bayreuther, K., Pfleiderer, G., 1989b. The differentiation of normal and transformed human fibroblasts in vitro is influenced by electromagnetic fields. Exp. Cell Res. 182, 610–621.
Roosterman, D., Goerge, T., Schneider, S.W., Bunnett, N.W., Steinhoff, M., 2006. Neuronal control of skin function: the skin as a neuroimmunoendocrine organ. Physiol. Rev. 86, 1309–1379.
Rowlatt, U., 1979. Intrauterine wound healing in a 20 week human fetus. Vichows Arch. A: Pathol. Anat. Histol. 381, 353–361.
Russell, S.B., Trupin, J.S., Kennedy, R.Z., Russell, J.D., Davidson, J.M., 1995. Glucocorticoid regulation of elastin synthesis in human fibroblasts: down-regulation in fibroblasts from normal dermis but not from keloids. J. Invest. Dermatol. 104, 241–245.
Sampurno, S., Cross, R., Pearson, H., Kaur, P., Malaterre, J., Ramsay, R.G., 2015. Myb via TGFbeta is required for collagen type 1 production and skin integrity. Growth Factors 33, 102–112.
Sanchez-Munoz, I., Granados, R., Holguin Holgado, P., Garcia-Vela, J.A., Casares, C., Casares, M., 2015. The use of adipose mesenchymal stem cells and human umbilical vascular endothelial cells on a fibrin matrix for endothelialized skin substitute. Tissue Eng., A 21, 214–223.
Sandulache, V.C., Zhou, Z., Sherman, A., Dohar, J.E., Hebda, P.A., 2003. Impact of transplanted fibroblasts on rabbit skin wounds. Arch. Otolaryngol.—Head Neck Surg. 129, 345–350.
Schafer, I.A., Kovach, M., Price, R.L., Fratianne, R.B., 1991. Human keratinocytes cultured on collagen gels form an epidermis which synthesizes bullous pemphigoid antigens and alpha 2 beta 1 integrins and secretes laminin, type IV collagen, and heparan sulfate proteoglycan at the basal cell surface. Exp. Cell Res. 195, 443–457.
Schafer, I.A., Pandy, M., Ferguson, R., Davis, B.R., 1985. Comparative observation of fibroblasts derived from the papillary and reticular dermis of infants and adults: growth kinetics, packing density at confluence and surface morphology. Mech. Ageing Dev. 31, 275–293.
Schneider, M.R., Schmidt-Ullrich, R., Paus, R., 2009. The hair follicle as a dynamic miniorgan. Curr. Biol. 19, R132–R142.
Schon, M., Rheinwald, J.G., 1996. A limited role for retinoic acid and retinoic acid receptors RAR alpha and RAR beta in regulating keratin 19 expression and keratinization in oral and epidermal keratinocytes. J. Invest. Dermatol. 107, 428–438.
Schönherr, E., Beavan, L.A., Hausser, H., Kresse, H., Culp, L.A., 1993. Differences in decorin expression by papillary and reticular fibroblasts in vivo and in vitro. Biochem. J. 290 (Pt 3), 893–899.
Schor, S.L., Schor, A.M., Rushton, G., Smith, L., 1985. Adult, foetal and transformed fibroblasts display different migratory phenotypes on collagen gels: evidence for an isoformic transition during foetal development. J. Cell Sci. 73, 221–234.
Sebastian, A., Allan, E., Allan, D., Colthurst, J., Bayat, A., 2011. Addition of novel degenerate electrical waveform stimulation with photodynamic therapy significantly enhances its cytotoxic effect in keloid fibroblasts: first report of a potential combination therapy. J. Dermatol. Sci. 64, 174–184.
Selekman, J.A., Grundl, N.J., Kolz, J.M., Palecek, S.P., 2013. Efficient generation of functional epithelial and epidermal cells from human pluripotent stem cells under defined conditions. Tissue Eng. C: Methods 19, 949–960.
Shannon, D.B., McKeown, S.T., Lundy, F.T., Irwin, C.R., 2006. Phenotypic differences between oral and skin fibroblasts in wound contraction and growth factor expression. Wound Repair Regen. 14, 172–178, official publication of the Wound Healing Society [and] the European Tissue Repair Society.
Sher, I., Zisman-Rozen, S., Eliahu, L., Whitelock, J.M., Maas-Szabowski, N., Yamada, Y., Breitkreutz, D., Fusenig, N.E., Arikawa-Hirasawa, E., Iozzo, R.V., Bergman, R., Ron, D., 2006. Targeting perlecan in human keratinocytes reveals novel roles for perlecan in epidermal formation. J. Biol. Chem. 281, 5178–5187.
Shevchenko, R.V., James, S.L., James, S.E., 2010. A review of tissue-engineered skin bioconstructs available for skin reconstruction. J. R. Soc. Interface 7, 229–258, the Royal Society.
Shin, Y.-H., Seo, Y.-K., Yoon, H.-H., Yoo, B.-Y., Song, K.-Y., Park, J.-K., 2011. Comparison of hair dermal cells and skin fibroblasts in a collagen sponge for use in wound repair. Biotechnol. Bioprocess E 16, 793–800.
Shiraha, H., Gupta, K., Drabik, K., Wells, A., 2000. Aging fibroblasts present reduced epidermal growth factor (EGF) responsiveness due to preferential loss of EGF receptors. J. Biol. Chem. 275, 19343–19351.
Simpson, C.L., Patel, D.M., Green, K.J., 2011. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat. Rev. Mol. Cell Biol. 12, 565–580.
Sivamani, R.K., Lam, S.T., Isseroff, R.R., 2007. Beta adrenergic receptors in keratinocytes. Dermatol. Clin. 25, 643–653, x.
Smithmyer, M.E., Sawicki, L.A., Kloxin, A.M., 2014. Hydrogel scaffolds as in vitro models to study fibroblast activation in wound healing and disease. Biomat. Sci. 2, 634–650.
Smola, H., Stark, H.J., Thiekotter, G., Mirancea, N., Krieg, T., Fusenig, N.E., 1998. Dynamics of basement membrane formation by keratinocyte-fibroblast interactions in organotypic skin culture. Exp. Cell Res. 239, 399–410.
Smola, H., Thiekotter, G., Baur, M., Stark, H.J., Breitkreutz, D., Fusenig, N.E., 1994. Organotypic and epidermal-dermal co-cultures of normal human keratinocytes and dermal cells: Regulation of transforming growth factor alpha, beta1 and beta2 mRNA levels. Toxicol. In Vitro 8, 641–650.

Smola, H., Thiekotter, G., Fusenig, N.E., 1993. Mutual induction of growth factor gene expression by epidermal-dermal cell interaction. J. Cell Biol. 122, 417–429.
Soma, T., Tajima, M., Kishimoto, J., 2005. Hair cycle-specific expression of versican in human hair follicles. J. Dermatol. Sci. 39, 147–154.
Sorensen, S., Solvsten, H., Politi, Y., Kragballe, K., 1997. Effects of vitamin D3 on keratinocyte proliferation and differentiation in vitro: modulation by ligands for retinoic acid and retinoid X receptors. Skin Pharmacol. 10, 144–152, the official journal of the Skin Pharmacology Society.
Sorrell, J.M., Baber, M.A., Caplan, A.I., 2004. Site-matched papillary and reticular human dermal fibroblasts differ in their release of specific growth factors/cytokines and in their interaction with keratinocytes. J. Cell. Physiol. 200, 134–145.
Sorrell, J.M., Baber, M.A., Caplan, A.I., 2007. Clonal characterization of fibroblasts in the superficial layer of the adult human dermis. Cell Tissue Res. 327, 499–510.
Sorrell, J.M., Baber, M.A., Caplan, A.I., 2008. Human dermal fibroblast subpopulations; differential interactions with vascular endothelial cells in coculture: nonsoluble factors in the extracellular matrix influence interactions. Wound Repair Regen. 16, 300–309.
Sorrell, J.M., Caplan, A.I., 2004. Fibroblast heterogeneity: more than skin deep. J. Cell Sci. 117, 667–675.
Sorrell, J.M., Caplan, A.I., 2009. Fibroblasts-a diverse population at the center of it all. Int. Rev. Cell Mol. Biol. 276, 161–214.
Sorrell, J.M., Carrino, D.A., Baber, M.A., Asselineau, D., Caplan, A.I., 1999. A monoclonal antibody which recognizes a glycosaminoglycan epitope in both dermatan sulfate and chondroitin sulfate proteoglycans of human skin. Histochem. J. 31, 549–558.
Stark, H.J., Baur, M., Breitkreutz, D., Mirancea, N., Fusenig, N.E., 1999. Organotypic keratinocyte cocultures in defined medium with regular epidermal morphogenesis and differentiation. J. Invest. Dermatol. 112, 681–691.
Stark, H.J., Boehnke, K., Mirancea, N., Willhauck, M.J., Pavesio, A., Fusenig, N.E., Boukamp, P., 2006. Epidermal homeostasis in long-term scaffold-enforced skin equivalents. The journal of investigative dermatology. Symp. Proc. 11, 93–105, the Society for Investigative Dermatology, Inc. [and] European Society for Dermatological Research.
Stark, H.J., Szabowski, A., Fusenig, N.E., Maas-Szabowski, N., 2004a. Organotypic cocultures as skin equivalents: A complex and sophisticated in vitro system. Biol. Proced. Online 6, 55–60.
Stark, H.J., Willhauck, M.J., Mirancea, N., Boehnke, K., Nord, I., Breitkreutz, D., Pavesio, A., Boukamp, P., Fusenig, N.E., 2004b. Authentic fibroblast matrix in dermal equivalents normalises epidermal histogenesis and dermoepidermal junction in organotypic co-culture. Eur. J. Cell Biol. 83, 631–645.
Steiling, H., Werner, S., 2003. Fibroblast growth factors: key players in epithelial morphogenesis, repair and cytoprotection. Curr. Opin. Biotechnol. 14, 533–537.
Stephens, P., Davies, K.J., Occleston, N., Pleass, R.D., Kon, C., Daniels, J., Khaw, P.T., Thomas, D.W., 2001a. Skin and oral fibroblasts exhibit phenotypic differences in extracellular matrix reorganization and matrix metalloproteinase activity. Br. J. Dermatol. 144, 229–237.
Stephens, P., Hiscox, S., Cook, H., Jiang, W.G., Zhiquiang, W., Thomas, D.W., 2001b. Phenotypic variation in the production of bioactive hepatocyte growth factor/scatter factor by oral mucosal and skin fibroblasts. Wound Repair Regen. 9, 34–43.
Stratakis, C.A., Mastorakos, G., Mitsiades, N.S., Mitsiades, C.S., Chrousos, G.P., 1998. Skin manifestations of Cushing disease in children and adolescents before and after the resolution of hypercortisolemia. Pediatr. Dermatol. 15, 253–258.
Strutz, F., Okada, H., Lo, C.W., Danoff, T., Carone, R.L., Tomaszewski, J.E., Neilson, E.G., 1995. Identification and characterization of a fibroblast marker: FSP1. J. Cell Biol. 130, 393–405.
Sundberg, C., Ljungstrom, M., Lindmark, G., Gerdin, B., Rubin, K., 1993. Microvascular pericytes express platelet-derived growth factor-beta receptors in human healing wounds and colorectal adenocarcinoma. Am. J. Pathol. 143, 1377–1388.
Supp, D.M., Boyce, S.T., 2005. Engineered skin substitutes: practices and potentials. Clin. Dermatol. 23, 403–412.
Supp, D.M., Hahn, J.M., Glaser, K., McFarland, K.L., Boyce, S.T., 2012. Deep and superficial keloid fibroblasts contribute differentially to tissue phenotype in a novel in vivo model of keloid scar. Plast. Reconstr. Surg. 129, 1259–1271.
Szabowski, A., Maas-Szabowski, N., Andrecht, S., Kolbus, A., Schorpp-Kistner, M., Fusenig, N.E., Angel, P., 2000. c-Jun and JunB antagonistically control cytokine-regulated mesenchymal-epidermal interaction in skin. Cell 103, 745–755.
Szpaderska, A.M., Walsh, C.G., Steinberg, M.J., DiPietro, L.A., 2005. Distinct patterns of angiogenesis in oral and skin wounds. J. Dent. Res. 84, 309–314.
Szpaderska, A.M., Zuckerman, J.D., DiPietro, L.A., 2003. Differential injury responses in oral mucosal and cutaneous wounds. J. Dent. Res. 82, 621–626.
Tajima, S., Pinnell, S.R., 1981. Collagen synthesis by human skin fibroblasts in culture: studies of fibroblasts explanted from papillary and reticular dermis. J. Invest. Dermatol. 77, 410–412.
Tan, J.Y., Sriram, G., Rufaihah, A.J., Neoh, K.G., Cao, T., 2013. Efficient derivation of lateral plate and paraxial mesoderm subtypes from human embryonic stem cells through GSKi-mediated differentiation. Stem Cells Dev. 22, 1893–1906.
Taylor, G., Lehrer, M.S., Jensen, P.J., Sun, T.T., Lavker, R.M., 2000. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. Cell 102, 451–461.
Thakoersing, V.S., Gooris, G.S., Mulder, A., Rietveld, M., El Ghalbzouri, A., Bouwstra, J.A., 2012. Unraveling barrier properties of three different in-house human skin equivalents. Tissue Eng. C: Methods 18, 1–11.
Thakoersing, V.S., van Smeden, J., Mulder, A.A., Vreeken, R.J., El Ghalbzouri, A., Bouwstra, J.A., 2013. Increased presence of monounsaturated fatty acids in the stratum corneum of human skin equivalents. J. Invest. Dermatol. 133, 59–67.
Theobald, V.A., Lauer, J.D., Kaplan, F.A., Baker, K.B., Rosenberg, M., 1993. "Neutral allografts"—lack of allogeneic stimulation by cultured human cells expressing MHC class I and class II antigens. Transplantation 55, 128–133.
Thornton, M.J., 2002. The biological actions of estrogens on skin. Exp. Dermatol. 11, 487–502.
Tobin, D.J., Gunin, A., Magerl, M., Handijski, B., Paus, R., 2003. Plasticity and cytokinetic dynamics of the hair follicle mesenchyme: implications for hair growth control. J. Invest. Dermatol. 120, 895–904.
Toma, J.G., Akhavan, M., Fernandes, K.J., Barnabe-Heider, F., Sadikot, A., Kaplan, D.R., Miller, F.D., 2001. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat. Cell Biol. 3, 778–784.
Tran, C.T., Huynh, D.T., Gargiulo, C., Nguyen, P.T., Tran, T.T., Huynh, M.T., Nguyen, T.T., Filgueira, L., Strong, D.M., 2011. In vitro culture of Keratinocytes from human umbilical cord blood mesenchymal stem cells: the Saigonese culture. Cell Tissue Banking 12, 125–133.
Truzzi, F., Marconi, A., Pincelli, C., 2011. Neurotrophins in healthy and diseased skin. Dermatoendocrinol 3, 32–36.
van den Bogaard, E.H., Bergboer, J.G., Vonk-Bergers, M., van Vlijmen-Willems, I.M., Hato, S.V., van der Valk, P.G., Schroder, J.M., Joosten, I., Zeeuwen, P.L., Schalkwijk, J., 2013. Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. J. Clin. Invest. 123, 917–927.
van Kilsdonk, J.W., van den Bogaard, E.H., Jansen, P.A., Bos, C., Bergers, M., Schalkwijk, J., 2013. An in vitro wound healing model for evaluation of dermal substitutes. Wound Repair Regen. 21, 890–896.
Varkey, M., Ding, J., Tredget, E.E., 2011. Differential collagen-glycosaminoglycan matrix remodeling by superficial and deep dermal fibroblasts: potential therapeutic targets for hypertrophic scar. Biomaterials 32, 7581–7591.
Varkey, M., Ding, J., Tredget, E.E., 2014a. Fibrotic remodeling of tissue-engineered skin with deep dermal fibroblasts is reduced by keratinocytes. Tissue Eng., A 20, 716–727.
Varkey, M., Ding, J., Tredget, E.E., 2014b. Superficial dermal fibroblasts enhance basement membrane and epidermal barrier formation in tissue-engineered skin: implications for treatment of skin basement membrane disorders. Tissue Eng., A 20, 540–552.
Verdier-Sevrain, S., Bonte, F., Gilchrest, B., 2006. Biology of estrogens in skin: implications for skin aging. Exp. Dermatol. 15, 83–94.
Vorsmann, H., Groeber, F., Walles, H., Busch, S., Beissert, S., Walczak, H., Kulms, D., 2013. Development of a human three-dimensional organotypic skin-melanoma spheroid model for in vitro drug testing. Cell Death Dis. 4, e719.
Wagner, I., Materne, E.M., Brincker, S., Sussbier, U., Fradrich, C., Busek, M., Sonntag, F., Sakharov, D.A., Trushkin, E.V., Tonevitsky, A.G., Lauster, R., Marx, U., 2013. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab Chip 13, 3538–3547.
Wang, J., Ding, J., Jiao, H., Honardoust, D., Momtazi, M., Shankowsky, H.A., Tredget, E.E., 2011. Human hypertrophic scar-like nude mouse model: characterization of the molecular and cellular biology of the scar process. Wound Repair Regen. 19, 274–285.
Wang, J., Dodd, C., Shankowsky, H.A., Scott, P.G., Tredget, E.E., Wound Healing Research, G., 2008. Deep dermal fibroblasts contribute to hypertrophic scarring. Lab. Invest. 88, 1278–1290, a journal of technical methods and pathology.
Werner, S., 1998. Keratinocyte growth factor: a unique player in epithelial repair processes. Cytok. Growth Factor Rev. 9, 153–165.
Werner, S., Grose, R., 2003. Regulation of wound healing by growth factors and cytokines. Physiol. Rev. 83, 835–870.
Werner, S., Krieg, T., Smola, H., 2007. Keratinocyte-fibroblast interactions in wound healing. J. Invest. Dermatol. 127, 998–1008.
Werner, S., Peters, K.G., Longaker, M.T., Fuller-Pace, F., Banda, M.J., Williams, L.T., 1992. Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc. Natl. Acad. Sci. U.S.A. 89, 6896–6900.
Werner, S., Smola, H., 2001. Paracrine regulation of keratinocyte proliferation and differentiation. Trends Cell Biol. 11, 143–146.
Werner, S., Smola, H., Liao, X., Longaker, M.T., Krieg, T., Hofschneider, P.H., Williams, L.T., 1994. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. Science (New York, N.Y.) 266, 819–822.
Whitby, D.J., Ferguson, M.W., 1991a. The extracellular matrix of lip wounds in fetal, neonatal and adult mice. Development 112, 651–668.
Whitby, D.J., Ferguson, M.W., 1991b. Immunohistochemical localization of growth factors in fetal wound healing. Dev. Biol. 147, 207–215.
Whitby, D.J., Longaker, M.T., Harrison, M.R., Adzick, N.S., Ferguson, M.W., 1991. Rapid epithelialisation of fetal wounds is associated with the early deposition of tenascin. J. Cell Sci. 99 (Pt 3), 583–586.
Whiting, D.A., 2001. Possible mechanisms of miniaturization during androgenetic alopecia or pattern hair loss. J. Am. Acad. Dermatol. 45, S81–S86.
Winiarska, A., Mandt, N., Kamp, H., Hossini, A., Seltmann, H., Zouboulis, C.C., Blume-Peytavi, U., 2006. Effect of 5alpha-dihydrotestosterone and testosterone on apoptosis in human dermal papilla cells. Skin Pharmacol. Physiol. 19, 311–321.

Wong, C.E., Paratore, C., Dours-Zimmermann, M.T., Rochat, A., Pietri, T., Suter, U., Zimmermann, D.R., Dufour, S., Thiery, J.P., Meijer, D., Beermann, F., Barrandon, Y., Sommer, L., 2006. Neural crest-derived cells with stem cell features can be traced back to multiple lineages in the adult skin. J. Cell Biol. 175, 1005–1015.

Wong, J.W., Gallant-Behm, C., Wiebe, C., Mak, K., Hart, D.A., Larjava, H., Hakkinen, L., 2009. Wound healing in oral mucosa results in reduced scar formation as compared with skin: evidence from the red Duroc pig model and humans. Wound Repair Regen. 17, 717–729.

Xia, W., Phan, T.T., Lim, I.J., Longaker, M.T., Yang, G.P., 2004. Complex epithelial-mesenchymal interactions modulate transforming growth factor-beta expression in keloid-derived cells. Wound Repair Regen. 12, 546–556.

Xu, X., Chen, C., Akiyama, K., Chai, Y., Le, A.D., Wang, Z., Shi, S., 2013. Gingivae contain neural-crest- and mesoderm-derived mesenchymal stem cells. J. Dent. Res. 92, 825–832.

Yamada, Y., Itano, N., Hata, K., Ueda, M., Kimata, K., 2004. Differential regulation by IL-1beta and EGF of expression of three different hyaluronan synthases in oral mucosal epithelial cells and fibroblasts and dermal fibroblasts: quantitative analysis using real-time RT-PCR. J. Invest. Dermatol. 122, 631–639.

Yamaguchi, Y., Itami, S., Tarutani, M., Hosokawa, K., Miura, H., Yoshikawa, K., 1999. Regulation of keratin 9 in nonpalmoplantar keratinocytes by palmoplantar fibroblasts through epithelial-mesenchymal interactions. J. Invest. Dermatol. 112, 483–488.

Younai, S., Venters, G., Vu, S., Nichter, L., Nimni, M.E., Tuan, T.L., 1996. Role of growth factors in scar contraction: an in vitro analysis. Ann. Plast. Surg. 36, 495–501.

Zhu, K.Q., Engrav, L.H., Gibran, N.S., Cole, J.K., Matsumura, H., Piepkorn, M., Isik, F.F., Carrougher, G.J., Muangman, P.M., Yunusov, M.Y., Yang, T.M., 2003. The female, red Duroc pig as an animal model of hypertrophic scarring and the potential role of the cones of skin. Burns 29, 649–664.

Zimmermann, D.R., Dours-Zimmermann, M.T., Schubert, M., Bruckner-Tuderman, L., 1994. Versican is expressed in the proliferating zone in the epidermis and in association with the elastic network of the dermis. J. Cell Biol. 124, 817–825.