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Review

Functional histopathology of keloid disease

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Summary. Keloid disease is a benign, yet locally aggressive and recurrent cutaneous fibroproliferative condition characterised by excessive scarring. Unique to humans, keloids represent the end-point of a spectrum of abnormal wound healing, are aesthetically disfiguring and can cause major functional impairment. Its heterogeneous phenotype can confound clinical diagnosis leading to mismanagement. This review examines the histological morphology of keloid disease relative to the underlying pathobiology, places it in the context of other cutaneous fibroses and highlights gaps within the literature that hinder differential diagnosis. The pathological similarity to hypertrophic scarring, dermatofibrosarcoma protuberans, dermatofibroma and scleroderma emphasise the importance of detailing the architectural and cellular components of this unique entity. In the papillary dermis keloid tumours show a tongue-like advancing edge that resembles invasive tumour growth. A thickened but flattened epidermis, hyalinised haphazardly arranged collagen bundles that dominate the dermis with subsequent obliteration of the papillary-reticular boundary along with displacement and eventually destruction of skin appendages, exemplify additional hallmark findings associated with keloid disease. Compared to healthy skin, keloid scars show an increased type I/III collagen ratio, decreased fibrillin-1 and decorin expression, increased dermal cellularity and increased expression of fibronectin, versican, elastin and tenascin in the reticular dermis and hyaluronan and osteopontin in the epidermis. We illustrate these "pathognomonic" features of keloid disease by representative micrographs and discuss them in the context of inflammation, hypoxia and tension - as key elements of keloid disease. Finally, we highlight deficits within the keloid research literature as well as discuss important areas for future research in keloid histology.

Key words: Keloid, Histopathology, ECM, Cutaneous fibroses

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Introduction

Keloid scars invariably arise following skin trauma that entails damage to both epidermis and dermis. While the pathogenesis of keloid disease (KD) remains incompletely elucidated, it does represent an abnormality of normal wound healing mechanisms (Butler et al., 2008; Gauglitz et al., 2011). Despite an

Abbreviations. BMZ, Basement membrane zone; DEJ, Dermo-epidermal junction; DFSP, Dermatofibrosarcoma protuberans; ECM, Extracellular matrix; EMI, Epithelial-mesenchymal interactions; EMT, Epithelial-mesenchymal transition; HA, Hyaluronic acid; HTS, Hypertrophic scar; IHC, Immunohistochemistry; KALT, Keloid associated lymphoid tissue; KD, Keloid disease; KF, Keloid fibroblasts; KK, Keloid keratinocytes; MC, Mast cell; NSF, Normal skin fibroblasts; NSK, Normal skin keratinocytes; PD, Papillary dermis; RD, Reticular dermis; SLRP, Small leucine rich protein; TGFβ, Transforming growth factor; αSMA, alpha smooth muscle actin

increased incidence in the third decade (Seifert and Mrowietz, 2009) and a higher preponderance among dark-skinned individuals (Sun et al., 2014), there are case reports describing keloid in different anatomical locations affecting humans of both genders, all ages (Tirgan et al., 2013) and ethnicities (Shih and Bayat 2012). KD has been described as an inability to restrain the wound healing process, resulting in an excess of scar tissue (Gauglitz et al., 2011). An imbalance between the phases of inflammation, proliferation and remodelling is thought to contribute to the distinguishing histologic features associated with KD and other cutaneous fibroses, such as hypertrophic scars, dermatofibrosarcoma protuberans (DFSP) and dermatofibroma. Despite significant recent progress in the pathobiology of KD (see for recent literature: (Suarez et al., 2013; Ogawa et al., 2014; Spiekman et al., 2014; Chen et al., 2015)) we have yet to identify the one pivotal pathway that determines keloid development, the course of KD and/or its response to therapy. The histological features associated with KD likely reflect a combination of distinct pathobiological elements, whose relative importance is likely to differ between affected individuals and which may underlie the heterogeneity that exists not only between and within keloid lesions but also between patients.

Much of keloid research to date has focussed on identifying potential biomarkers for KD associated with the classical four phases of wound healing (haemostasis, inflammation, proliferation and remodelling). These integrated phases of wound healing are regulated by numerous transcription factors, cytokines and growth factors, which are beyond the scope of this review (see: (Gurtner et al., 2008; Muller et al., 2012; Ding and Tredget, 2014; Haertel et al., 2014; Olczyk et al., 2014). By approaching the histology from a pathobiological angle that compares KD with normal wound healing one can attempt to understand the dynamic histological features of KD and can correlate them with the emerging advances in molecular keloid research (Arbi et al., 2015).

Written from a pathobiological perspective, this review focuses on the histology of KD, emphasising its cellular and extracellular matrix (ECM) components and how these allow one to distinguish KD from similar fibrotic cutaneous conditions. Whilst this review attempts to facilitate differential diagnosis, we also highlight gaps within the KD literature and delineate promising areas for future research in this field.

Haemostasis (Day 1)

As the first phase of wound healing, the initial formation of a haemostatic clot may not be considered histologically relevant in KD, where the clinical criteria for diagnosis require lesions to be present for at least a year. However, the cytokine release from de-granulated platelets e.g. platelet-derived growth factor, stimulate surrounding fibroblasts to lay down early ECM and through chemotaxis, are responsible for much of the inflammatory infiltrate seen in histological sections (Weyrich et al., 2009). Additionally, it has been hypothesised that inadequate removal of the fibrin clot in keloid, secondary to PAI-1 excess, may lead to sustained fibroblastic release of collagen and result in fibrosis (Simone et al., 2014).

Inflammation

"Appropriate inflammation" is an essential part of the normal wound healing mechanism (Guo and Dipietro, 2010) but persistence of this process can lead to many of the features observed in KD. It has been hypothesised that the sustained release of cytokines and growth factors from immune cells results in continued cell proliferation and ECM deposition (Reinke and Sorg, 2012). The presence, even in mature keloid scars, of an inflammatory infiltrate (Fig. 1A), keloid-associated lymphoid tissue (KALT) and excess matrix, supports this theory. Immunohistochemistry has demonstrated the persistence of a number of pro-inflammatory immune cells, primarily macrophages and lymphocytes, known to be involved in chronic inflammation. Also discussed here, as well as schematically depicted in Fig. 5, are cells with an immune role shown to be present in keloid tissue when compared with normal skin, including mast cells, Langerhans cells and fibrocytes (antigen presenting function).

Macrophages

These cells, derived from circulating monocytes, are a heterogeneous population dependent on their mode of activation. Macrophages can be classically (M1, CD68+) or alternatively (M2, CD163+) activated and both of these are present within the keloid PD and RD. At both intralesional and perilesional sites, these cells were increased in comparison with normal skin and normal skin scars (Bagabir et al., 2012). The classically activated macrophages are degradative and therefore are responsible for much of the destruction of the ECM. Additionally, macrophages can produce collagen (usually collagen VII) and may also secrete perlecan and versican (Schnoor et al., 2008). These cells have been found to lie in close contact with both other immune cells and fibroblasts suggesting that, through their release of cytokines, these immune cells may be engaged in a paracrine loop resulting in the evident histological changes (Shaker et al., 2011).

Lymphocytes

Immune cell infiltrate in keloid tissue was shown by Martin and Muir to demonstrate that T-cell lymphocytes were present in higher numbers over a longer period of time when compared with normal scar tissue. Additionally, a more abundant T-cell population, particularly Th cells, was noted at the margin of the

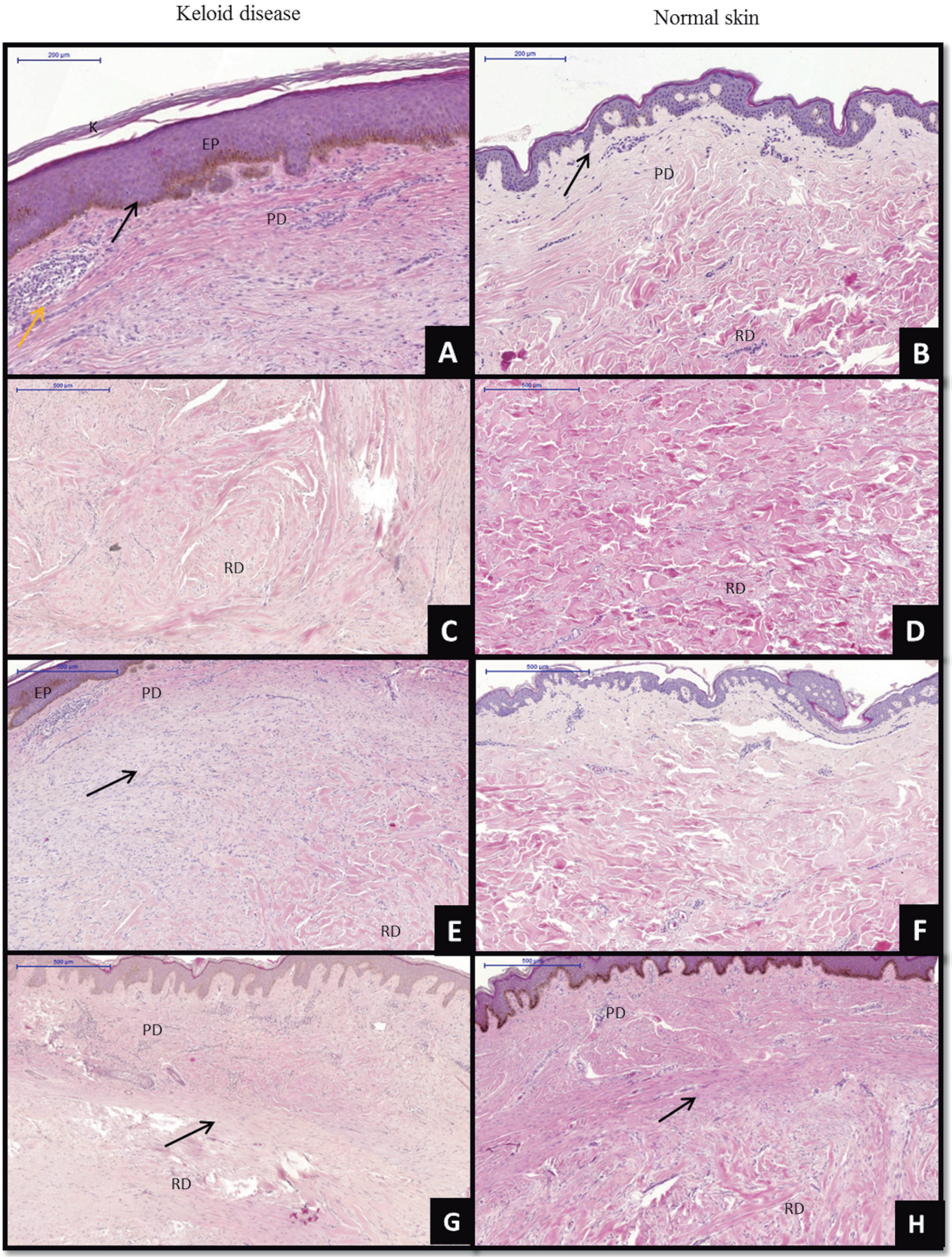

Fig. 1. Morphological analysis of keloid and normal skin using haematoxylin and eosin (H&E). The tissue was formalin-fixed and paraffin-embedded before cutting 5µm sections and stained using a standard protocol. **A.** Thickened flattened epidermis of keloid (black arrow) with inflammation (orange arrow). Evidence of hyperkeratosis (K). **B.** Normal skin with thin epidermis and rete ridges. **C.** Whorls of haphazard hyalinised thickened collagen in keloid. **D.** Organised fine collagen of normal skin. **E.** Increased cellularity in keloid (black arrow). **F.** Reduced cellularity in normal skin. **G and H.** Horizontal fibrous band in upper dermis of keloid (black arrow).

keloid tissue when compared with the less active centre (Martin and Muir, 1990). Lymphocytes are morphologically identified as small round cells with round nuclei but immunohistochemistry is required to reliably differentiate between B and T cells. T-cell lymphocytes, typically those that are CD3+, are consistently found to be increased in keloid tissue, mostly perivascularly, but also dispersed between

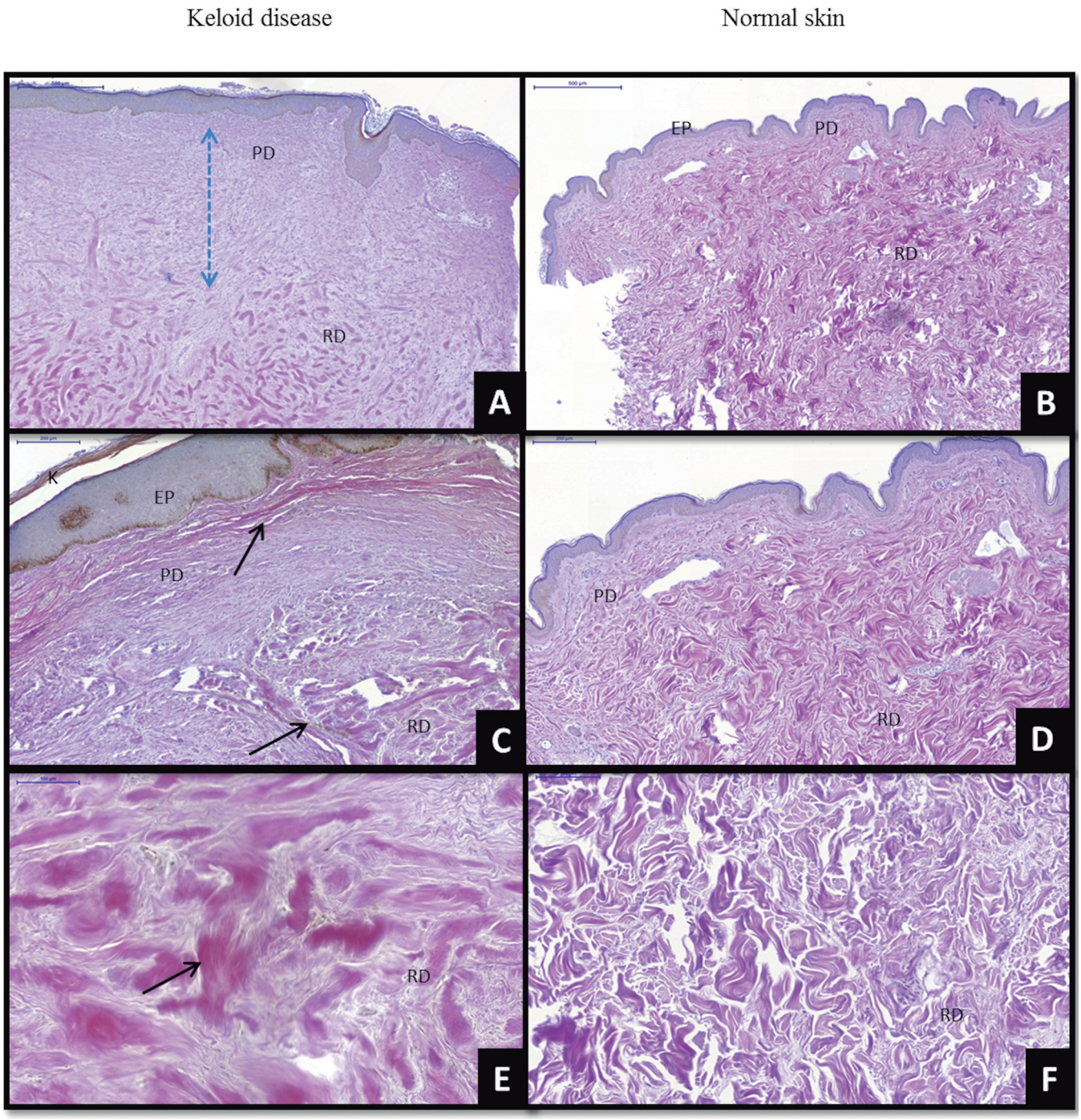

Fig. 2. Morphological analysis of keloid and normal skin using Herovici staining. **A and B.** Larger volume of sub-epidermal area with increased Collagen III:I ratio vs normal skin (blue arrows). **C and D.** Transition from thin sub-epidermal Collagen I to thick coarse Collagen I with intervening collagen III vs normal skin. **E and F.** Thickened haphazard collagen I:III ratio vs normal skin.

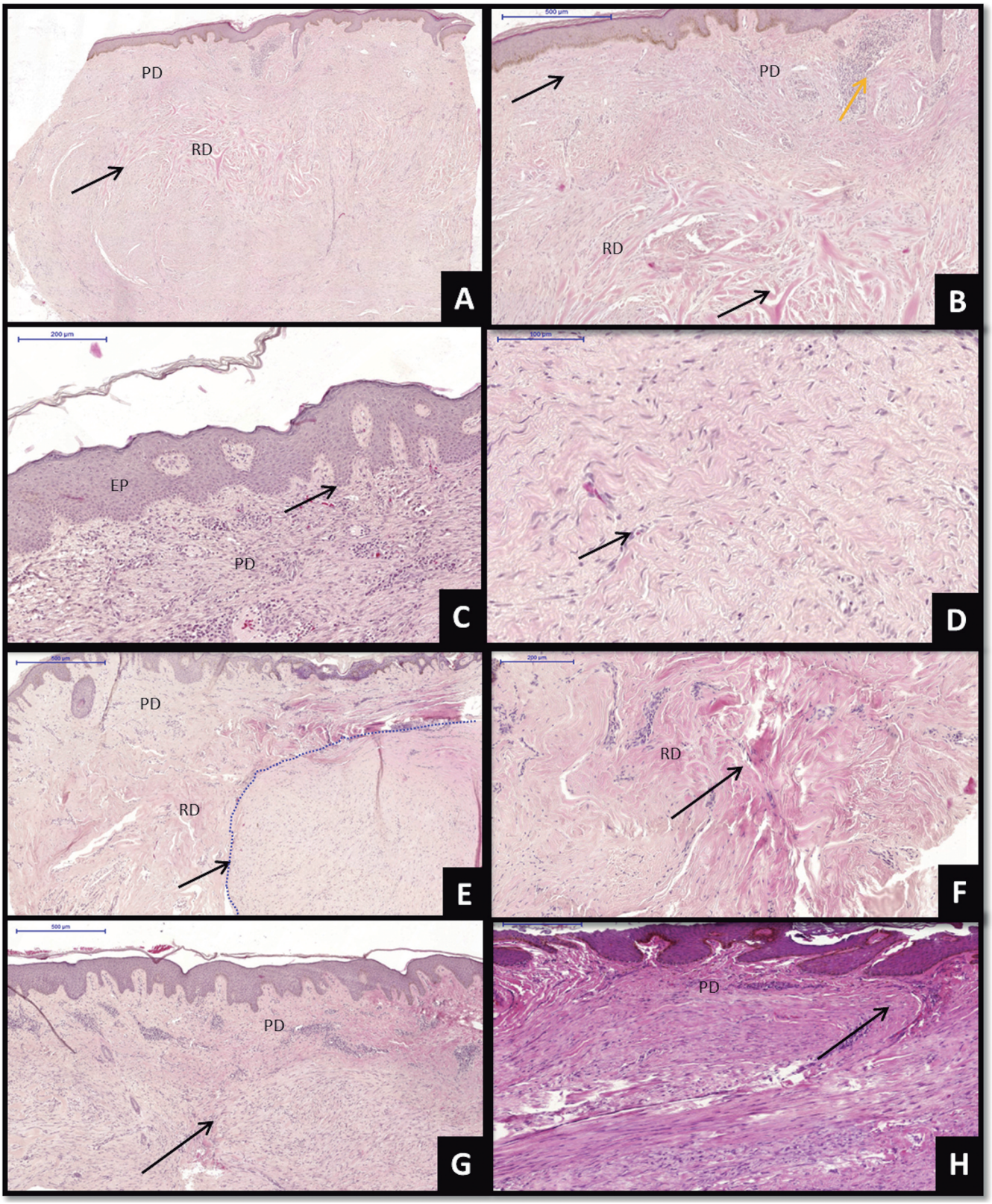

Fig. 3. H&E staining of keloid tissue demonstrating specified features. **A.** Obliteration of the papillary-reticular boundary. **B.** Combination of multiple features including inflammation (orange arrow) fine to coarse collagen fibres (black arrows), hyper-cellularity and thickened flattened epidermis. **C.** Thickened epidermis but not flattened. **D.** Finer collagen but remains hyper-cellular. **E.** Nodule within dermis. **F.** Focal point of collagen explosion. **G and H.** Advancing edge within papillary dermis as shown by black arrows.

collagen bundles (Moshref and Mufti, 2010; Shaker et al., 2011). There has also been a reported increase in the CD4+:CD8+ ratio within T lymphocytes in keloid samples, however the significance of this remains undetermined (Boyce, 1994; Bagabir et al., 2012). One study looked at 28 hypertrophic and 26 keloid samples, determining there was a delayed-type immune reaction that seemed to be age-dependent in hypertrophic scars, maintaining the possibility to differentially diagnose these lesions (Santucci et al., 2001). While T lymphocytes invariably showed increased numbers in keloid tissue when compared with normal skin or normal scar, the distribution of B-cell lymphocytes was more erratic. Many of the earlier studies reported few B lymphocytes in either normal or scarred dermis (Martin and Muir, 1990; Boyce, 1994), however more contemporary works, using the pan-B cell marker CD20, have identified a higher number at both central and marginal sites when compared with controls (Shaker et al., 2011; Bagabir et al., 2012). Interestingly, a recent study identified novel patterns of KD-associated inflammatory infiltrates that resemble tertiary lymphoid follicles and has coined this "keloid-associated lymphoid tissue" (KALT). Despite being present in only some of the KD samples examined, the presence of aggregates emphasises the importance of an immune role in KD and supports the need for further investigation (Bagabir et al., 2012). Perhaps this phenomenon shows some similarities to the most recently described perivascular clustering of leukocytes that may be essential for the elicitation of effective contact hypersensitivity responses in murine skin (Natsuaki et al., 2014).

Mast cells (MC)

Found in large numbers in a study including 44 keloid samples, MC were evidenced to be in close contact with fibroblasts, a term referred to as "cell talk" (Shaker et al., 2011). These cells release histamine when de-granulated, which may be responsible for the pruritus

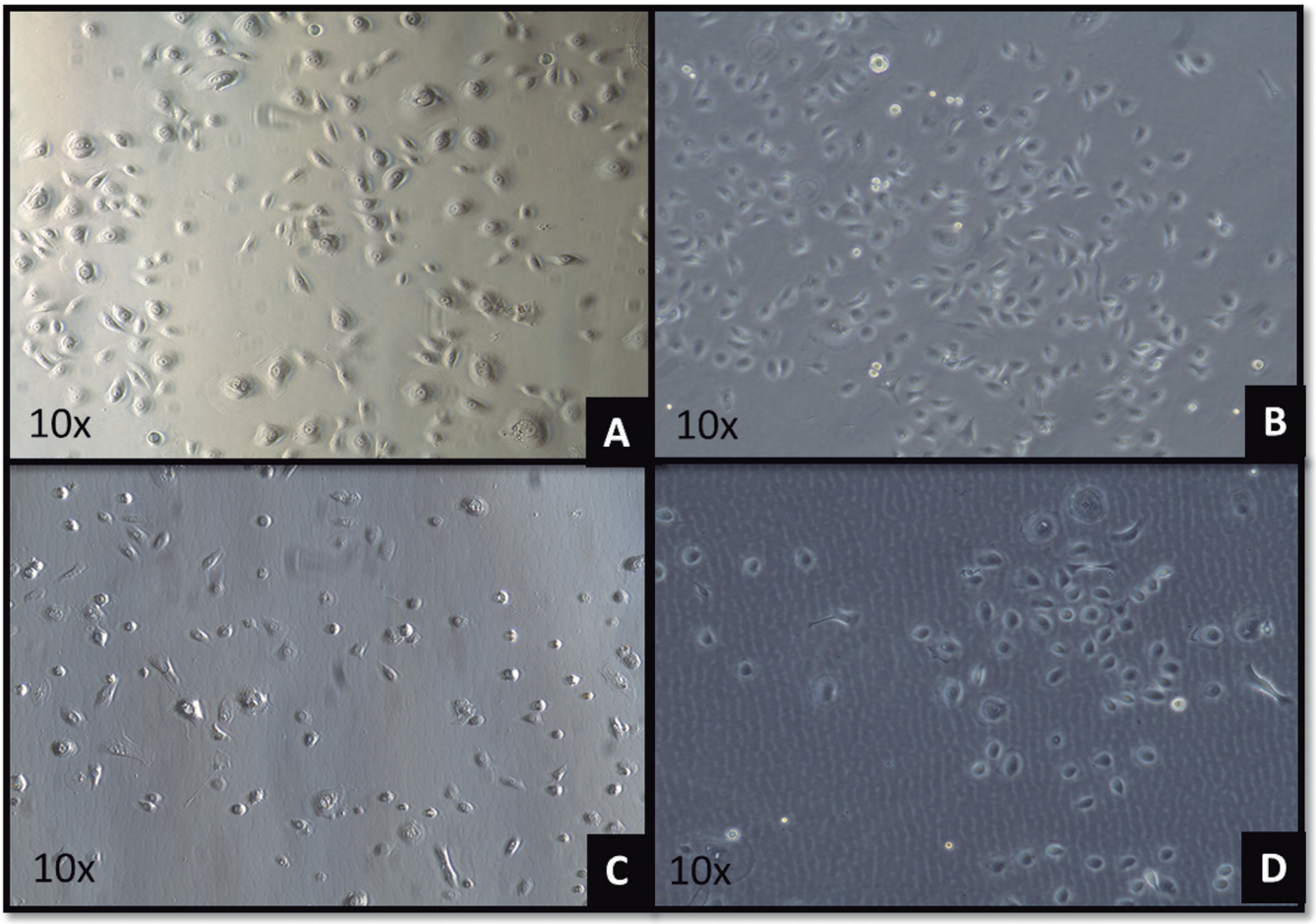

Fig. 4. Keloid and normal keratinocytes in monolayer *in vitro* culture P0. **A and C.** keloid keratinocytes form looser colonies than their normal skin counterparts **(B and D)**.

and erythema associated with keloids, explaining why these symptoms are attenuated with the application of corticosteroid and/or compression (Lavker and Schechter, 1985; Hassel et al., 2007; Schneider et al., 2013). When normal scar and hypertrophic scar tisssue were analysed, it was found that the MC numbers increased with increasing scar age, although it was highlighted that the activation of these cells and not just the number of cells was of significance (Niessen et al., 2004). Several studies have shown that MC are increased in keloid tissue (Kamath et al., 2002; Ammendola et al., 2013; Dong et al., 2014). A recent study showed an increased number of mature and activated MC, both intralesionally and perilesionally in keloid tissue when compared with normal skin and normal scars (Bagabir et al., 2012). Direct cell-cell contact between MC and KF has recently been shown using transmission electron microscopy and was hypothesised to be responsible for KF proliferation through the MC release of cytokines and growth factors. This group also postulated that increased collagen may attract MC, which then reduce collagen bulk through phagocytosis (Arbi et al., 2015).

Langerhans cells

Despite the finding that the release of IL-1a and attraction of T lymphocytes by activated Langerhans cells can influence collagen levels, there is limited evidence in the literature on their abnormal presence in keloid tissue (Niessen et al., 2004). One study, using CD207 staining, found no difference in numbers between keloid and normal skin or normal scars (Bagabir et al., 2012). A separate group using anti-CD1a showed the presence of these cells in hypertrophic scars diminished over time finding reduced cells positive in old hypertrophic scars but did show positive staining present in approximately 40% of keloid tissue samples (Santucci et al., 2001).

Fibrocytes

Fibrocytes are mesenchymal precursor cells expressing myeloid (CD45RO) and haematopoietic (CD34) antigens, as well as structural proteins including collagen I, collagen III, fibronectin and vimentin (Abe et al., 2001; Bucala, 2012). Morphologically distinguishable by their spindle shape and mid-length fibre-like projections, these cells may account for up to 10% of cells infiltrating wound sites (Bucala et al., 1994).

Fibrocytes have been shown to be a population distinct from MSC (mesenchymal stem cells), identified by a double positive CD34/collagen I and/or CD45/collagen I stain (Iqbal et al., 2012). Discussed here because of their their pro-angiogenic and immune role (Reilkoff et al., 2011), acting as antigen-presenting cells, fibrocytes also contribute to both the proliferation and remodelling phases of wound healing through differentiation into fibroblast and myofibroblast populations (Bellini and Mattoli, 2007).

Although less dense than normal fibroblasts within keloid tissue (0.4 versus 4.8 per area (Ueda et al.,

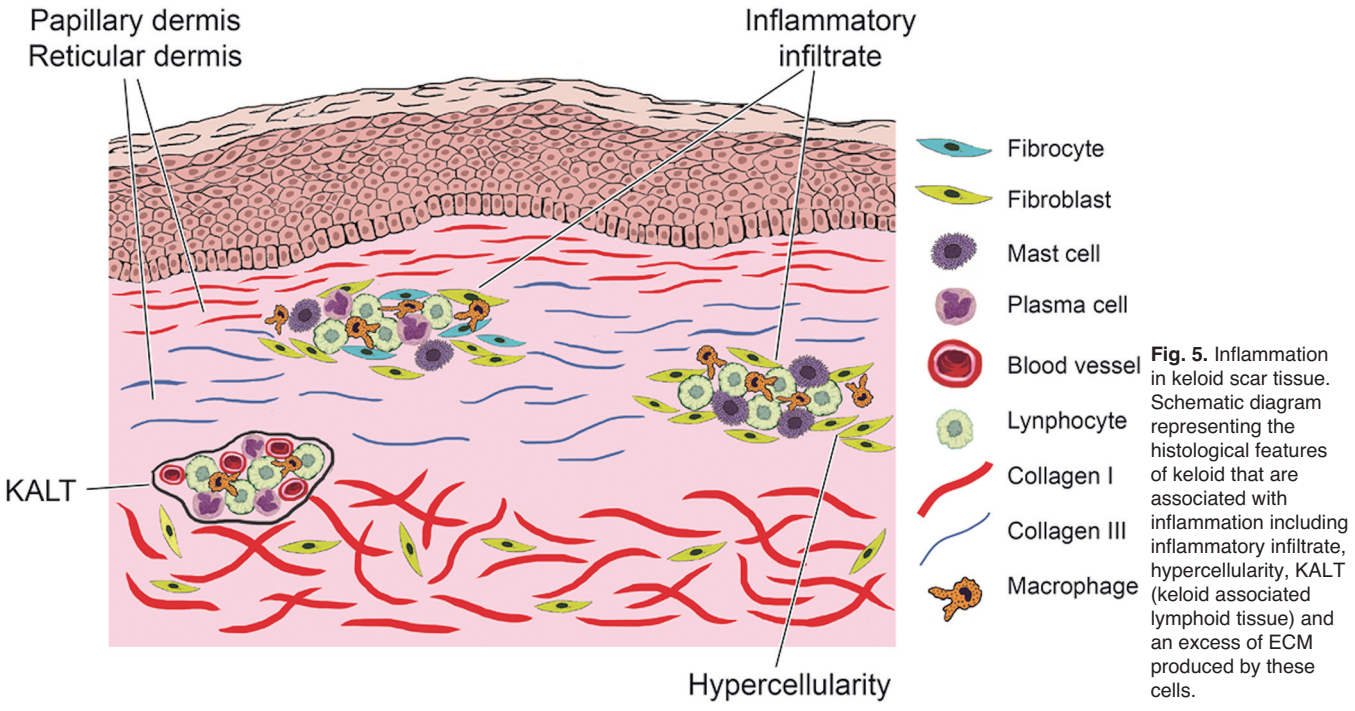

Fig. 5. Inflammation in keloid scar tissue. Schematic diagram representing the histological features of keloid that are associated with inflammation including inflammatory infiltrate, hypercellularity, KALT (keloid associated lymphoid tissue) and an excess of ECM produced by these cells.

1999)), fibrocytes are thought to contribute to excess scarring through collagen production, both themselves and by induction of fibroblasts, as well as by differentiation into αSMA-expressing myofibroblasts, which exert contractile forces on the healing wound (Iqbal et al., 2012). Fibrocytes have been shown to affect keratinocyte proliferation and impact the re-epithelialisation process (Kao et al., 2011) and are postulated to be the source of scarring in burn wounds, where it may be difficult for fibroblasts to migrate from the healthy wound edge (Mathangi Ramakrishnan et al., 2012).

This, in addition to their increased prolyl-4-hydroxylase (Aiba and Tagami, 1997) release, an enzyme responsible for the stabilisation of the collagen triple helix, is thought to result in the excess ECM deposition that is the hallmark of KD (McCoy et al., 1980; Ala-Kokko et al., 1987). As fibrocytes are derived from CD14+ cells in the peripheral blood (Curran and Ghahary 2013), these have been used as an upstream target for therapeutics. Serum amyloid P (SAP) inhibits fibrocyte differentiation thereby decreasing the myofibroblast population in the wound and reducing scarring (Naik-Mathuria et al., 2008; Blakaj and Bucala, 2012). Although not the primary source of collagen, it may be that targeting fibrocytes is an important strategy for targeting the scar tissue volume in KD.

Proliferation

This wound healing phase is marked by the formation of granulation tissue, re-epithelialisation, neo-angiogenesis and new ECM deposition (Baum and Arpey, 2005). While the features associated with these processes are discussed under proliferation, there is significant overlap with both the inflammatory and remodelling phases whereby cells, cytokines and growth factors from all three stages contribute to the dysregulation leading to keloid histology (Shih et al., 2010).

It is postulated that a prolonged proliferation phase is responsible for the majority of features considered characteristic of KD (Young et al., 2013) and these are schematically depicted in Fig. 6. While an overproduction of collagen is the subject of much focus in the literature, it is the microscopic changes in the non-collagenous ECM elements that distinguish keloid from other cutaneous fibroses and are therefore discussed in further detail.

Epidermal proliferation

The majority of literature to-date has described the keloid epidermis as thickened with flattened rete ridges (Fig. 1A,B), presumably secondary to pressure from the large collagen bundles occupying the dermis that impinge upon the epidermis (Lee et al., 2004; Kose and Waseem, 2008). The normal epidermis, composed mostly of keratinocytes in distinct stages of differentiation is also populated by Merkel cells, Langerhans cells, T lymphocytes (CD4+, CD8+ or γδ-T cell receptor+) and melanocytes, complemented by intraepidermal nerve fibres arising from distally located, extracutaneous sensory neurons arising from spinal dorsal root ganglia (Kanitakis, 2002; Fradette et al., 2003; Di Meglio et al., 2011).

Although not extensively studied, keloid epidermis exhibits an increased immune cell infiltrate (discussed

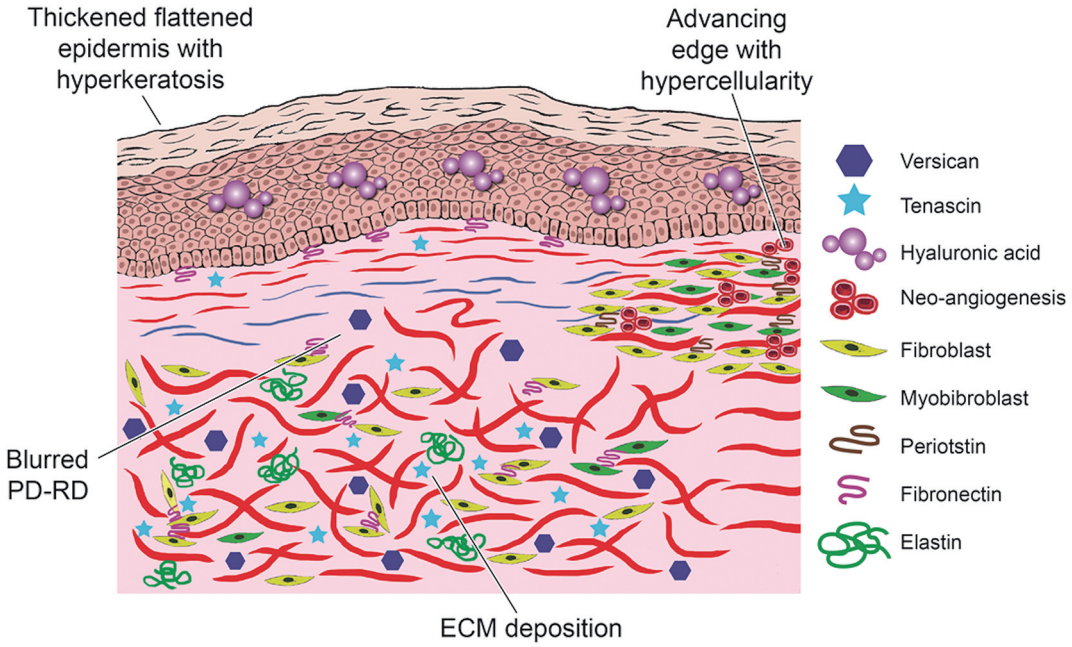

Fig. 6. Proliferation in keloid scar tissue. Schematic diagram representing the histological features of keloid disease associated with proliferative stage of wound healing. Hypercellularity resulting in both thickened epidermis and an advancing edge in the dermis. Increased ECM deposition resulting in blurring of the papillary-reticular boundary. ECM molecules known to show increased staining in keloid tissue are depicted here.

below) at both the lesion centre and margin (Bagabir et al., 2012) as well as positively expressing immune cell mediators, COX-1 and COX-2 (Abdou et al., 2014). The keloid epidermis expresses osteopontin, although this was not compared with normal skin (Miragliotta et al., 2014), positively stained compared with normal skin for TGFβ-1 (Abdou et al., 2011) and no difference was shown between KD, HTS and normal skin with regard to epidermal insulin-like growth factor-1 receptor staining (Hu et al., 2014). There is however, a noticeable paucity of information in the literature on melanocytes and Merkel cells in keloid epidermis and any abnormalities in these cell types compared to the epidermis of healthy skin or normal scars.

Keratinocytes

Keratinoctyes change their morphology with differentiation status, allowing formation of a stratified epithelium that generates a protective barrier (Eckert, 1989). Under certain pathological conditions, epidermal keratinocytes express alternate keratins to those found in normal skin. This is also seen in KD, where hyper-proliferation marker keratin 16 has been shown to be expressed as well as keratin 2e, normally found in the cornified envelope but in keloid, it is expressed in the basal epidermis (Bloor et al., 2003; Ong et al., 2010). Histochemically this goes along with epidermal hypergranulosis and hyperkeratosis (Figs. 1A, 2C) (Moshref and Mufti, 2010).

This keloid keratinocyte (KK) hyper-proliferation is thought to account for the consistently thickened epidermis observed in keloid histology and may contribute to BMZ changes. With most of the histological disorganisation occurring below the DEJ, the basal cells in keloid tissue appear regular with minimal disarray, albeit showing some vacuolar changes (Moshref and Mufti, 2010). Whilst previously thought only to be a result of aberrations in dermal tissue, namely fibroblasts, the effect of local paracrine loops involving keratinocytes and the epidermis has now been realised in the context of cutaneous wound healing and scar formation (Garner, 1998; Machesney et al., 1998).

3D models and co-cultures have been employed to demonstrate dysregulation of EMI in keloid formation (Lim et al., 2001, 2002) and investigate keloid pathology (Supp et al., 2012; van den Broek et al., 2014). Additionally, KF expression was shown to be altered when in direct cell-cell contact with keratinocytes when compared with exposure only to keratinocyte medium (Funayama et al., 2003). As well as their autocrine and paracrine roles in initiating inflammatory responses (Pasparakis et al., 2014), keratinocytes participate in regulation of fibroblast proliferation, apoptosis and collagen production, thus participating in ECM synthesis (Kose and Waseem, 2008; Wang et al., 2015).

While KK have been compared to normal skin keratinocytes (NSK) on a transcriptional level (Xia et al., 2006) there is limited evidence in the literature comparing their monolayer cell morphologies. One study implicates this cell's role in EMT by showing immunohistochemical evidence of desmosomal discontinuity impacting keratinocyte adhesion. They also describe KK displaying detached, more widely disbanded colonies when compared with NSK and showed evidence of faster migration using a scratch assay (Hahn et al., 2013). We also found that NSK formed tighter, more compact colonies when compared with KK in monolayer culture as shown in Fig. 4.

Melanocytes

While there are a few studies that investigated the expression of melanocytic factors in KF, including melanocortin-1 receptor (Luo et al., 2013) and propiomelanocortin (Teofoli et al., 1997), the only reference to keloid tissue pigmentation was from that of one grafted into a hamster cheek pouch, where they hypothesised that grafted keloid tissue must contain melanoblasts that differentiated into melanocytes in the

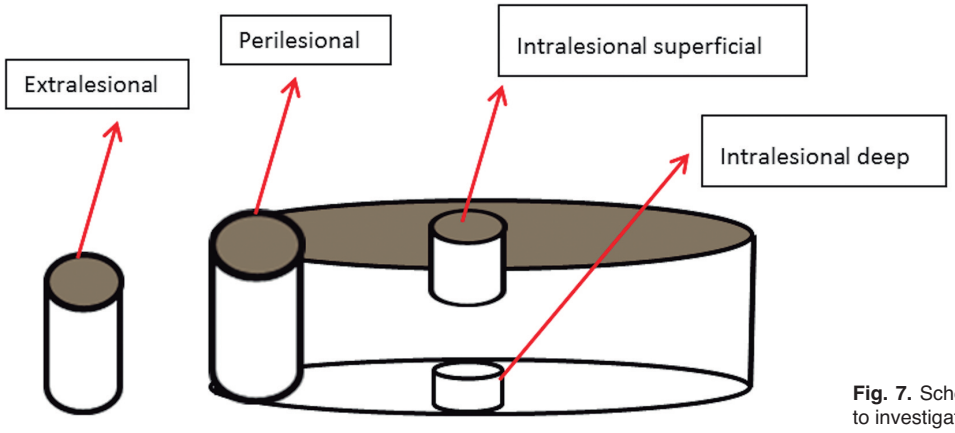

Fig. 7. Schematic representation of defined sites used to investigate site-specific keloid disease pathobiology.

hamster environment, something that does not occur in the human samples (Hochman et al., 2005). There is some evidence that melanocytes induce fibroblast proliferation resulting in increased ECM deposition (Gao et al., 2013) and that reduction in melanocytes may be responsible for the relative success of current therapies (Har-Shai et al., 2006). A recent study investigating the effect of melanocytes and fibroblasts on the contractile behaviour of keratinocytes suggested that the balance of melanocytes rather than the presence or absence is more significant (Rakar et al., 2014). Given the recent experimental findings in mice, that wounding or UV trauma of the epidermis recruits melanocyte stem cells from the hair follicle epithelium into the epidermis (Chou et al., 2013) and the concept that melanocytes may play a more active role in damage response and tissue remodelling than widely appreciated (Paus, 2013), there is clearly a lot of scope for further research for defining the role of the melanocyte in keloid pathobiology.

Dermal proliferation

Extracellular matrix deposition

The dermis of human skin is composed of two distinct layers: papillary and reticular. It has been noted in several histological keloid specimens that, as a result of matrix overproduction, the distinction between the papillary and reticular dermis becomes blurred (Fig. 3A) (Huang et al., 2012).

Collagen

Collagen, an abundant triple helix protein (Mienaltowski and Birk, 2014), is the main ECM constituent and is found in abundance in KD. It is generally accepted that keloids contain surplus amounts of collagen within the dermis, accounting for most of the bulk of the scar tissue. Lee et al. in 2004 referred to this haphazard collagen pattern (Fig. 1C) as "keloidal collagen", identifying it as a histological hallmark of KD, albeit with a low sensitivity (Lee et al., 2004). The discord regarding the exact quantity, type, morphology and location of this collagen is due to much of the literature being based on individual findings, often using small sample numbers or lacking comparisons to normal and hypertrophic scars.

These factors, compounded by the inherent heterogeneity of keloid fibrosis, have led to confusion in diagnosis and inappropriate management strategies (Atiyeh et al., 2005). There is little disagreement over the "keloidal collagen" being thickened, hyalinised and eosinophilic with a distinct glassy appearance. Whether confined to a particular zone (Bux and Madaree, 2010) or diffusely distributed throughout the dermis (Moshref, 2010), the collagen bundles have been described as organised parallel to the surface (Ehrlich et al., 1994) but more frequently haphazardly arranged (Knapp et al., 1977; Da Costa et al., 2008). These bundles occur with higher frequency and increased thickness in the perilesional sub-papillary (Bux and Madaree, 2010) or RD when compared with intralesional and extralesional sites (Syed et al., 2011).

Furthermore, it has been described that the ratio of the two primary collagens involved in wound healing, types I and III, is altered in keloids (Abergel et al., 1985) with more recent studies supporting an elevated collagen I/III ratio (Fig. 2) based on raised collagen I and unaltered collagen III levels (Uitto et al., 1985; Peltonen et al., 1991) . This ratio may vary between different sites within the keloid tissue as demonstrated in a recent study revealing increased collagen I and III production within perilesional sites of the keloid, both *in vitro* and *in vivo*, when it is compared with intralesional and extralesional sites (Syed et al., 2011).

In addition to "keloidal collagen" several other features allow one to distinguish keloid from hypertrophic scarring (Lee et al., 2004). These are summarised in Table 4. The most significant of these was the presence of a PD tongue-like advancing edge, which has also been described in relation to keloid histology previously and may be a point of differentiation from hypertrophic scars (Fig. 3G,H) (Cosman and Wolff, 1972; Moshref and Mufti, 2010).

The expression of other collagens in keloid tissue, such as collagen V, collagen VI and collagen VII, remains to be systematically analysed. Additionally the absence of collagen nodules, originally believed to be a diagnostic marker of KD (Linares and Larson, 1974; Ehrlich et al., 1994), cannot be safely used to exclude KD, as several studies have identified their presence in both keloid (Fig. 3E) and hypertrophic scars (Kischer and Brody, 1981; Lee et al., 2004).

Non-collagenous matrix

The role of the ECM is both structural and regulatory, requiring a balanced composition to maintain optimal structure and function (Mitts et al., 2010). Composed of varying amounts of glycosaminoglycans, proteoglycans and elastic fibres, a disturbance in the proportions of these molecules can result in excess matrix, consistent with the formation of raised dermal scarring. Microfibrillar proteins constitute the bulk of the non-collagenous ECM, along with hyaluronan and fibronectin. Deposited in early natal life, microfibrillar proteins, consisting of an elastin core and surrounding fibrillin microfibrils, extend from the DEJ through the papillary dermis (PD), where they are thin, to the reticular dermis (RD), where they form thick bands (Kielty, 2006; Kadoya et al., 2015). Microfibrillar proteins also influence cell migration and adhesion through sequestration and presentation of wound healing cytokines such as TGFβ (Ramirez and Rifkin, 2009; Massam-Wu et al., 2010).

In KD, immunohistochemistry, stereology and multiphoton microscopy have shown that elastin and

fibrillin are disorganised when compared with normal skin and normal scar tissue. Fibrillin-1 deposition is decreased throughout the tissue (Amadeu et al., 2004) whereas elastin is almost absent in the PD yet significantly increased in the RD, where it forms nodes (Chen et al., 2011). It has been speculated that this may result from an initial overproduction of both collagen and elastin by KF, followed by the continued presence of collagen without elastin.

Hyaluronan (HA), a glycosaminoglycan thought to be of central importance to scarless fetal wound healing (Namazi et al., 2011), is a significant ECM component. Hyaluronan has structural and regulatory roles as well as implications in angiogenesis and inflammation (Frenkel, 2014). It has been postulated to be important in effective epithelialisation and may influence fibroblast morphology (Tan et al., 2011), thus securing itself and the molecules involved in its synthesis and degradation as potential targets in keloid therapeutics (Sidgwick et al., 2013).

In keloids, HA has been shown to exhibit a different expression pattern from that of normal and hypertrophic scarring: using biotinylated hyaluronic acid binding protein (HABP), normal skin, normal scar tissue and to a lesser degree hypertrophic scars showed HA to be principally concentrated in the PD and yet scanty in the epidermis (Bertheim and Hellstrom, 1994). In contrast, keloids consistently demonstrated the opposite phenomenon, with staining reduced in the PD and being maximal in the intercellular space between keratinocytes in the suprabasal layers of the epidermis (Meyer et al., 2000; Tan et al., 2011). On this basis, HA alone has been used to classify scar types for the purpose of experimentation (Hellstrom et al., 2014).

Fibronectin, a linking glycoprotein that binds to integrins and other matrix molecules, forming an early component of granulation tissue (Martino et al., 2011; To and Midwood 2011), is more strongly expressed in keloid than normal tissue (Kischer and Hendrix, 1983; Ashcroft et al., 2013) and can be further enhanced by TGFβ1 and abrogated by triamcinolone acetonide treatment (Lee et al., 2013). While some studies describe diffuse fibronectin staining in both normal skin and keloid (Knaggs et al., 1994), those that yielded more intense staining in keloid tissue describe it as increased at the DEJ and co-localised with fibroblasts between collagen bundles in the dermis (Sible et al., 1994). KF also produce more fibronectin in culture than normal dermal fibroblasts (Babu et al., 1989). During normal wound healing with respect to the above, keloid fibronectin is gradually replaced by neo-dermis; notably however its expression is maintained in abnormal scarring (Santucci et al., 2001). This continued presence may result in prolonged interaction with other matrix proteins or cells forming the bulky growth that is typical of KD. If the keloid lesion can be confirmed to stain positive for fibronectin, reducing fibronectin expression might be therapeutically beneficial (different ways of reducing fibronectin expression have already been reported) (Lee et al., 2011; Liang et al., 2013).

Besides these larger and better-investigated molecules, little is known in the literature on the histology of ECM in KD, such as dermatopontin, periostin, small leucine-rich proteoglycans (SLRP) and tenascin (Sidgwick and Bayat, 2012). The latter, a hexameric glycoprotein involved in fibrosis, has been shown to have some homology with fibronectin and potentially influence its biologic activity, similarly disappearing from the wound with the replacement of granulation tissue during normal wound healing (Shrestha et al., 1996; Halper and Kjaer, 2014). Having been previously associated with scleroderma (Lacour et al., 1992), hyper-proliferative skin conditions (Schalkwijk et al., 1991) and acne-associated KD (Knaggs et al., 1994), the role of tenascin was investigated in KD. This protein was diffusely expressed in keloid tissue, especially the RD, when compared with normal skin where it formed a linear band at the DEJ but failed to show any deeper positive staining (Dalkowski et al., 1999). This was supported in a more recent study where reduction in the overexpression of tenascin was seen using cryotherapy (Abdel-Meguid et al., 2014).

The ECM component dermatopontin has been implicated in wound re-epithelialisation (Krishnaswamy and Korrapati, 2014), delayed healing (Krishnaswamy et al., 2014) and fibrosis (Kuroda et al., 1999), although little is known on its role in KD. Due to the decreased expression of dermatopontin in KF (Russell et al., 2010), it was directly compared to leiomyomas, which also showed reduced dermatopontin mRNA expression in microarray analysis; the authors further reported that dermatopontin protein expression is also reduced in keloid tissue compared to healthy skin (Catherino et al., 2004).

As the most abundant glycoprotein in normal skin dermis, the SLRP decorin binds to other matrix proteins including collagen, fibronectin and thrombospondin, playing a role in ECM assembly and therefore an attractive protein to investigate in wound healing (Tracy et al., 2014). Decorin is thought to interact with dermatopontin to alter TGFβ expression, thereby affecting collagen fibrillogenesis (Zhang et al., 2006) and has been implicated in the inflammatory process of wound healing, involving toll-like receptors 2 and 4 (Merline et al., 2011), which have also been investigated in pathological scarring (Wang et al., 2011; Chen et al., 2013). Decorin has been shown to be comparable in normal skin and mature scars but reduced in early wound healing and delayed in abnormal wound healing such as post-burn hypertrophic scars (Scott et al., 1995; Sayani et al., 2000). Keloid immunohistochemistry showed no difference when compared with normal skin (Tan et al., 1993; Hunzelmann et al., 1996), although proteoglycan composition analysis by a more recent study reported decreased decorin expression in keloid versus normal skin (Carrino et al., 2012). This finding is supported by two recent studies that showed up-regulation of decorin in treated versus untreated keloid

samples (Trisliana Perdanasari et al., 2014; Chen et al., 2015). Interestingly, this molecule has recently been shown to be reduced in the tumour microenvironment when compared with normal tissue controls and therefore of potentially significant importance in a tumour suppression role (Neill et al., 2012; Bozoky et al., 2014). Decorin's postulated role (namely, to control TGFβ1 activity, thus manipulating collagen bundle formation) (Okamoto et al., 1999) along with the delayed appearance of decorin in hypertrophic scarring (Sayani et al., 2000) suggests there is a period in early keloid formation, where decorin administration or up-regulation could be therapeutically beneficial.

Analysis of biglycan, another SLRP, has generated contradictory results in the literature. In normal skin, biglycan descriptions range from no presence at all (Scott et al., 1995) to a linear band adjacent to the BMZ. In comparison, keloid tissue reportedly showed either indistinguishable staining from normal skin (Tan et al., 1993) or positive expression in the collagen nodules of the dermis, encouraging the theory that biglycan is associated with collagen deposition (Hunzelmann et al., 1996).

Periostin has been investigated in KD with regard to it's role in promoting angiogenesis (Zhang et al., 2015), a histological feature associated with this scar (Huang et al., 2012). Keloid tissue shows increased staining in both epidermis and dermis when compared with normal skin (Zhou et al., 2010) and significant co-localisation with CD31, suggesting a correlation with blood vessel density (Zhang et al., 2015). In hypertrophic scarring, periostin has been shown to increase dermal fibroblast

Table 1. Summary of the ECM molecules previously investigated in keloid disease tissue.

ECM molecule	Location within keloid tissue	Technique	Ref.
Collagen I	Abundant expression throughout dermis	Histochemistry (Masson's trichome)	Kauh et al., 1997
	↑ thicker bundles at margin, especially reticular dermis	Histochemistry (Herovici)	Syed et al., 2011
Collagen III	Thinner vs collagen I, ↑ in papillary dermis of keloid margin	Herovici	Syed et al., 2011
	Strongly ↑ in keloid vs normal skin	IHC	Naitoh et al., 2001
Collagen IV	Along BMZ and ↑ proximal to small blood vessels	IHC	Naitoh et al., 2001
Collagen VI	Co-localised with col I proximal to small blood vessels Also ↑ papillary dermis	IHC	Peltonen et al., 1991
Fibronectin	↑ at dermo-epidermal junction Co-localised with cells in deep dermis between collagen bundles	IHC	Sible et al., 1994
	Intense localisation with fibroblasts upper reticular	IHC	Kischer and Hendrix, 1983
	Diffuse positivity in keloid tissue	IHC	Knaggs et al., 1994; Santucci et al., 2001; Liang et al., 2013
Hyaluronan (HA)	Gross HA stain in upper layers epidermis PD- mesh-like staining, RD- Intense staining	HABP	Bertheim and Hellstrom, 1994
	↑ Intercellular in spinous & granular layers ↓HA in keloid dermis	HABP	Meyer et al., 2000
	↓ intensity stain in papillary dermis	HABP	Tan et al., 2011
Elastin	↓ superficial dermis ↑deep dermis, parallel to collagen	IHC Histomorphometric	Amadeu et al., 2004
	↓ elastic fibres all scar types	Verhoeff van Giesson stain	Kamath et al., 2002
	↓ elastic fibres, due to impaired fibrillin-1	IHC	Ikeda et al., 2009
	↑ elastin deep dermis, node structure	Multiphoton microscopy	Chen et al., 2011
Fibrillin	↓ superficial and deep dermis Thin fibres, no candelabra pattern	IHC Histomorphometric	Amadeu et al., 2004
	Altered distribution, thick irregular bundles	IHC	Ikeda et al., 2009
	Altered fibrillin distribution, related to TGFβ	IHC	Nie et al., 2008
Tenascin	Diffusely expressed in dermis Associated with ↑ collagen bundles in reticular dermis	IHC	Dalkowski et al., 1999
Dermatopontin	↓ stain compared with normal skin	IHC	Catherino et al., 2004
Decorin	Indistinguishable from normal skin, strong stain in dermis, weaker in epidermis	IHC	Tan et al., 1993; Hunzelmann et al., 1996; Catherino et al., 2004
Biglycan	↑ in nodular areas of keloid	IHC	Hunzelmann et al., 1996
	Indistinguishable from normal skin	IHC	Tan et al., 1993
Periostin	↑ in epidermis and dermis vs normal skin Co-localisation with CD31	IHC	Zhang et al., 2015
	↑ especially in acellular node region of deep dermis	IHC	Zhou et al., 2010
Versican	Intense deposition in keloid but not normal skin	IHC	Yagi et al., 2012

proliferation and differentiation into myofibroblasts (Crawford et al., 2015), but this has yet to be investigated in KD.

Finally, versican is a large proteoglycan capable of sequestering large amounts of water through its glycosaminoglycans and therefore, similar to HA, is theorised to be responsible for some of the volume in keloid scars (Yagi et al., 2013). Moreover, in the hair follicle mesenchyme, versican expression is correlated with inductive properties of specialised fibroblasts that engage in intimate EMI with the adjacent epithelium (Kishimoto et al., 1999; Soma et al., 2005; Ohyama et al., 2010). Versican immunostaining has revealed intense deposition in keloid tissue when compared with normal skin (Yagi et al., 2012) and this was confirmed in a separate protein study using composite gels (Carrino et al., 2012).

Fibroblasts

Fibroblasts are elongated spindle-shaped cells, defined by their ability to secrete ECM. They are a heterogeneous cell type, differing in function and morphology even within an organ (Sorrell and Caplan, 2009). Although identified morphologically, the varied fibroblast subtypes or derivatives express specific proteins that can be used to differentiate them within tissues. It has been shown that KF differ from NSF in ECM production, especially collagen, which is produced in excess in keloid scars (Lim et al., 2002). While there has been some debate in the past over the degree of cellularity in keloid tissue, the consensus is in favour of a high cellularity, with the predominance of those cells being fibroblasts (Shaker et al., 2011).

It has been noted that in addition to the haphazard collagen deposition, the fibroblasts themselves also lie in a disorganised fashion (Lee et al., 2004) and are frequently degenerate or necrotic (Bux and Madaree, 2010). Further to this, the cell phenotype can be heterogeneous within the scar itself as well as between scar types. It has been shown that the superficial (papillary) and deep (reticular) fibroblasts within keloid exhibit differential expression and are postulated to result in alternative keloid phenotypes clinically (Supp et al., 2012). These altered phenotypic KF respond differently to the NSF, not only in relation to cytokines and growth factors but also to immunomodulatory treatments (Russell et al., 1995). Other than phenotype, a key factor in the production of excess matrix appears to be the interaction of fibroblasts with both keratinocytes and the immune cell infiltrate (Martin and Muir, 1990; Shaker et al., 2011). Additionally, the fibroblasts of non-keloid tissue have been shown to respond in a keloid-like manner to the media taken from KF (Ashcroft et al., 2013).

Vascularity

Opinion diverges with regard to the vascularity of keloid scars. Studies have been conducted using

Table 2. Summary of the cells identified in keloid disease, their morphology and stain used to identify them within the tissue.

Cell type	Morphology	Stain	Present/Absent in KD	Sample no.	Ref.
Keratinocyte	Differentiation status dependent	Cytokeratins	K2e/K16 Present in epidermis (K6/K16 also present in HTS)	n=14	Machesney et al., 1996
				n=10	Bloor et al., 2003
				n=10	Ong et al., 2010
Langerhans cell	Dendritic suprabasal cells, 2% epidermis	CD1a, S-100, Langerin (CD207)	Present within epidermis	n=26	Santucci et al., 2001
				n=25	Bagabir et al., 2012
Melanocyte	Highly dendritic, basal epidermal	Fontana-masson, Mel-5	Present/increased within epidermis	n=1 into fragments	Hochman et al., 2005
Fibrocyte	Spindled, mid-length fibre-like projections	CD34/Coll I; CD45/Coll I; CD86	Present within dermis	n=11	Iqbal et al., 2012
Fibroblast	Spindle-shaped	TE-7, Fibronectin, vimentin	Increased activity within dermis	n=12 n=39	Theoret et al., 2013; Chong et al., 2015
Myofibroblast	Spindled, fusiform indented nuclei	αSMA, transgelin, cytoglobin, P4Hβ	Present within dermis	n=40	Lee et al., 2004
				n=26	Santucci et al., 2001
Mast cell	Large, mononuclear, Metachromatic granules	CD117, anti-chymase, anti-tryptase, c-kit	Increased in dermis	n=5	Dong et al., 2014
				n=25	Bagabir et al., 2012
Lymphocyte	Spherical/ovoid, densely packed nuclear chromatin dominating cytoplasm	Giemsa/wright, CD45 T: CD3, CD4&8, TCR B: CD20, CD38, CD79a	Present in epidermis & dermis, ?increased in both	n=25	Bagabir et al., 2012
				n=8	Boyce et al., 2001
Macrophage	Large with granules & vacuoles vs monocytes	CD68 (M1) CD163 (M2)	Increased in reticular & papillary dermis	n=25	Bagabir et al., 2012
				n=44	Shaker et al., 2011
Endothelial cell	Elongated, flat & aligned in direction blood flow	CD31, VEGF, vWf	Present in epidermis & dermis	n=15	Zhang et al., 2015
				n=9	Kischer et al., 1982

stereological analysis of dermal vessels (Amadeu et al., 2003), transmission electron microscopy (Ueda et al., 2004), doppler assessment and quantitative microscopic examination in combination with CD31, CD34, CD105, αSMA and VEGF immunostaining. While some of the literature favours hypervascularity associated with long and dilated vessels (Amadeu et al., 2003), the bulk of studies investigating keloid blood supply found limited microvasculature associated with luminal occlusion (Beer et al., 1998; Bux and Madaree, 2010; Har-Shai et al., 2011), most frequently attributed to obstruction by endothelial cells (Kischer et al., 1982; Kischer, 1992). The impaired blood supply within the keloid tissue has encouraged the hypothesis that hypoxia is a key element in the pathogenesis of KD (Butler et al., 2011). Bux and Madaree proposed that the impaired vasculature explains the features of degeneration and necrosis evident in keloid tissue and that the capillary occlusion results from chronic inflammation, occurring predominantly at the level of the subpapillary plexus (Bux and Madaree, 2010).

Due to the site-specific differences observed clinically (Syed et al., 2011), between keloid centre and margin, a more recent paper examined the differences in vascular density between these sites of the keloid lesion. Whilst there was no significant vascular density differences, it was noted that the vessels located centrally were more flattened, based on major and minor axes (Kurokawa et al., 2010). This correlated with the histological finding of an advancing edge with increased cellularity and microvasculature compared with the occluded vessels of the centre.

Remodelling

This phase of wound healing, in contrast to the relatively short preceding phases, can last for months to years and is thought to be delayed in keloid. The ultimate role of this phase is to increase the tensile strength within the wound, decrease the thickness of the newly formed tissue and promote terminal differentiation of the epidermis, thereby restoring a functional barrier (Carlson and Longaker, 2004). The role of the myofibroblast, which begins with contraction during the phase of proliferation, is instrumental in this phase and the failure to undergo apoptosis likely to be causative in keloid scar formation.

Myofibroblasts

Myofibroblasts are mesenchymal cells expressing characteristics of both fibroblasts and smooth muscle cells. The origin of this hybrid cell has long been under debate, with reports of differentiation from pericytes, fibroblasts, smooth muscle cells and most recently from fibrocytes (Hinz et al., 2012). Myofibroblasts, like their predecessors, are spindle-shaped cells but have fusiform indented nuclei, a fibronectin-rich but laminin-deplete layer at their surface and, in addition to expressing fibronectin and vimentin, express α smooth muscle actin (αSMA) (Eyden et al., 2009). It is these non-muscle myosin microfilaments in combination with gap junctions that allow participation in wound closure and may cause the contracture postulated to be causative in hypertrophic and keloid scarring (Van De Water et al., 2013; Tholpady et al., 2014).

There has been much controversy over whether there are myofibroblasts in KD (Matsuoka et al., 1988) and whether there is αSMA positive staining (Sarrazy et al., 2011). It had been suggested that αSMA is used as a differentiation marker between keloid and hypertrophic scars (Ehrlich et al., 1994), however it was confirmed that keloid cells do express αSMA (Lee et al., 2012) and due to the variability in expression in both forms of scarring (45% of keloid and 70% hypertrophic), this cannot be a reliable method of distinction (Lee et al., 2004).

Other markers have been used to detect myofibroblasts in keloid including transgelin, cytoglobin and prolyl-4-hydroxylase β, interestingly all of which are controlled by hypoxia, theorised to be one of the driving forces in keloid pathogenesis (Har-Shai et al., 2011). In fact, it has been reported that myofibroblasts are the predominant cell type present in keloid tissue regardless of the age of the lesion and that the collagen nodules in particular stain positive for αSMA (Santucci et al., 2001; Hunasgi et al., 2013). It may be that the αSMA expression of hypertrophic scars declines over

Table 3. Summary of the features of other skin-related fibrotic disorders in common with and different from keloid disease as well as the stains most commonly used in the diagnosis.

Condition	Histological features in common with KD	Histological features different from KD	Stain
Hypertrophic scar	Raised scar; Thickened collagen Nodules Increased cellularity	Non-flattened epidermis; Organised collagen fibres No recurrence	H&E αSMA
DFSP	Slow-growing; Raised, pigmented skin Recurrence	Increased nodularity; Honeycomb pattern Non-polarizing collagen	Vimentin; αSMA CD34+; XVIIIa-; S100-
Dermatofibroma	Thickened epidermis; Hyperkeratosis Hyalinised collagen	Scaly lesions; No recurrence Reduced cellularity; Grenz zone	XVIII+ CD34-
Scleroderma/ morphea	Pigmented Lack of adnexal appendages Nodules	Reduced cellularity; Collagen arranged parallel Systemic features	CD34-; CD1a; CD3; CD8; CD20+; CD25, CD57+

time whereas that of keloids remains constant, allowing for distinction of older lesions (Santucci et al., 2001; Sarrazy et al., 2011). This failure to quiesce or apoptose may account for the continued growth observed in keloids as myofibroblasts in normal wound healing disappear when re-epithelialisation is complete (Hinz, 2007; Darby et al., 2014; Li-Tsang et al., 2015).

Differential diagnose: KD versus other cutaneous fibroses

Since there is not one reliable and definitive keloid biomarker available, one needs to rely on both clinical appearance and histopathology in order to distinguish KD from other forms of cutaneous fibrosis (Table 3).

Hypertrophic scarring

The condition most commonly confused with keloid and a source of much contention is hypertrophic scarring. For the purposes of diagnosis and indeed research, several macroscopic criteria are generally applied to distinguish these two entities. Keloid scars extend beyond the margins of the original wound to invade the surrounding normal skin, whereas hypertrophic scars remain confined to the boundaries of the initial injury but push them out by expansion. This invasion is described histologically, by the advancing edge dominating the papillary dermis (Fong et al., 1999; Lee et al., 2004) but also as a clinically evident advancing edge (Cosman and Wolff, 1972). Unlike keloids, hypertrophic scars tend to regress with time and do not usually recur after excision but are more associated with contractures than their counterparts, due to a higher rate of fibrin matrix gel contraction (Mustoe et al., 2002). Keloid scars are more likely to be erythematous and pruritic compared to hypertrophic scars but both can present with these symptoms and indeed it happens that there may be a mixture of the two processes occurring within the one wound. This clinical pattern led to the description of keloids as having an inflammatory zone, at the lesion base, a raised pale central area and a regressing portion where it resembles normal scarring (Seifert and Mrowietz, 2009).

Dermatofibrosarcoma protuberans

Dermatofibrosarcoma protuberans (DFSP), more commonly confused with dermatofibroma, can also pose a diagnostic dilemma when considering keloid, especially in its early stages. Similar to keloid, this cutaneous sarcoma is slow-growing, absent from the hands and feet, has a higher incidence in darker skin (Criscione and Weinstock, 2007), occurs most frequently between 20-50 years of age and may recur on excision (Sabater-Marco et al., 2006). It differs macroscopically in that it is often larger and more nodular and in some instances has been shown to metastasize (Liang et al., 2014). Microscopically DFSP is discernible by a characteristic storiform pattern of spindle cells surrounded by fibrous stroma that causes a honeycomb pattern when it extends into adipose tissue (Sabater-Marco et al., 2006). Similar to keloid and several other fibroses, it has been shown to stain positive for both vimentin (Tsai et al., 2014) and occasionally positive, although more routinely negative, for αSMA (De Pasquale et al., 2009; Sundram, 2009; Kim et al., 2012).

Two markers have been used to specifically

Table 4. Summary of the characteristic features of keloid disease and their frequency, including data based on the images shown in Figs. 1-3.

Ref	Sample no.	Epidermis	Advancing edge	Collagen	Cellularity	Horizontal fibrous band	Inflammation	aSMA(+)	Vascularity
Moshref and Mufti, 2010	15	10/15 rete	-	15/15 haphazard	10/15	14/15	-	5/15	Sub-epidermal
Bux and Madaree, 2010	58	-	-	Thick bundles	Between collagen bundles. Fibroblastic. Immune	-	Chronic	-	Impaired angiogenesis
Santucci et al., 2001	26	Flattened. Adnexae displaced.	-	25/26 Thick; Hyalinised Haphazard	24/26 Diffuse, myofibroblasts	-	Persistent immune cell infiltrate	21/26	-
Lee et al., 2004	40	37/40 flattened	14/14 marginal sections	24/40 Thick hyalinised bundles	33/40	10/40	39/40 Lymphocytes 8/40 sinus tract/ ruptured follicle	18/40 (>10% +)	Disarray
Ong et al., 2010	10	Thickened	Lined with BM	-	-	-	-	-	-
Bayat et al., 2014 (unpublished)	13	Thickened & flattened 11/13 Thickened not flattened 2/13 Hyperkeratotic 12/13	3/13	Whorls, thickened Haphazard 11/13 Nodule 1/13 Fine, organised 1/13 Obliteration PD-RD 11/13	Diffuse 13/13 Including between collagen bundles	5/13 Upper dermis	10/13 most commonly sub-epidermally	-	10/13 occlusion PD-RD between collagen fibres, Neoangiogenesis deeper dermis

discriminate DFSP from other cutaneous pathologies: non-polarizable collagen (Barr et al., 1986) and positive $CD34^+$ staining (Aiba et al., 1992). There is a single reference to the polarization of keloid collagen that suggests that it is polarizable (Barr and Stegman, 1984), which may make it a potential point of differential diagnosis but this requires more studies before being reliably diagnostic. While the literature suggests consistent positive staining for CD34 in DFSP, the consensus in keloid seems to be that the perilesional and extralesional dermal sites are positive relative to the inflammatory process (Iqbal et al., 2010; Bakry et al., 2014). The CD34 immuno-staining in KD may show an inverse correlation with collagen I production (Aiba and Tagami, 1997).

Dermatofibroma

Like keloid, dermatofibroma often occurs at sites of previous trauma and microscopically appears ill-defined and frequently characterised by a hyperkeratotic, hyperplastic epidermis. Indeed, there is a keloid variant of this lesion that contains hyalinised collagen at the periphery leading to dependence on the presence of other features associated with dermatofibroma to rule out keloid (Alves et al., 2014). Dermatofibroma can be a scaly lesion more frequently occurring on the limbs, it tends not to recur following excision and in combination with microscopic features of a grenz zone (spared PD), elongated rete ridges and increased hair follicle structures, it should be distinguishable from keloid (Luzar and Calonje, 2010). The differences in PD at the margin of both lesions may be the diagnostic crux, where in keloid, it is the active site with increased cellularity and contrastingly in dermatofibroma, the PD is spared and the margin RD is the site of collagen bundles. Unlike DFSP, dermatofibroma (fibrous histiocytoma) is more easily differentiated by immunohistochemistry from DFSP than from keloid. A lesion that is CD34+ and factor XVIIIa negative is likely to be DFSP, however CD34- and XVIIIa positive is more likely to be dermatofibroma (Altman et al., 1993) although the jury is out with regard to keloid. Similar to CD34, there is a lack of uniformity within the literature with respect to factor XVIIIa staining in keloid, with studies claiming both absence and augmentation (Kamath et al., 2002; Onodera et al., 2007).

Morphea

Another condition frequently misdiagnosed is cutaneous scleroderma or morphea, which similar to dermatofibroma has a keloid variant, making it more difficult to distinguish from the classic keloid scar. Macroscopically, sclerosis is characterised by thickened dermis and occasionally nodules or keloid-like lesions that are hyper-pigmented and lack appendages. Microscopically these nodules consist of collagen bundles lying parallel to the dermis and reduced fibroblasts (Rencic et al., 2003). The presence of myofibroblasts has been suggested as a method to determine the stage of differentiation along a hypothetical continuum from morpheiform nodule to hypertrophic scar and finally keloid (Barzilai et al., 2003). These nodules can resemble keloid with flattened rete ridges, increased collagen and immune infiltrate (Buechner et al., 1993), diffuse tenascin staining (Lacour et al., 1992) and recently identified increased cartilage oligomeric matrix protein (COMP) staining (Moinzadeh et al., 2013). Due to the impact of systemic disease, it is essential to correctly diagnose these nodules and rule out any other signs of systemic sclerosis.

Perspectives for future, histopathology-based research into KD pathobiology

Site-specific disease

Based originally on the clinical differences observed between the margin and centre of keloid scars, site-specific variations have only recently been exploited as a way to potentially target the active site of disease. Macroscopically, the centre (intralesional) is often pale, soft and involuted when compared with the margin (perilesional), where there is frequently a raised erythematous edge considered to be the aggressive site of activity. It has also been shown that fibroblasts from the PD and RD behave differently (Supp et al., 2012), leading to division of the upper and lower centre of the lesion as two separate sites.

This novel approach has already shown altered behaviour on a molecular level with regard to apoptosis (Lu et al., 2007; Seifert et al., 2008), collagen expression (Syed et al., 2011) and also on a protein level (Javad and Day, 2012). While there are some histological studies that analysed these areas separately (Bagabir et al., 2012) the benefits of site-specific staining to aid diagnosis and allow targeted therapy has yet to be fully explored. The standardised dissection of each keloid lesion into defined sites (see, for example Fig. 7) and the examination of ECM component histology for each site would abrogate the risk of incorrect diagnosis based on sampling site.

Morphological classification

Despite the disparity between different studies in the literature with regard to keloid histopathology, there is consistent reference to the heterogeneity that exists concerning this entity. Macroscopically, the keloid scar varies from a flat, claw-like invading lesion to a polypoid pedunculated lesion, both with varying degrees of central regression and marginal erythema and firmness. As these details are often not recorded and correlated with the histological findings, it is difficult to confidently identify effective treatments or judge accurate prognoses. Although keloid is usually the end-point in scar scale classification (Mustoe et al., 2002), it

would be beneficial to draw up a classification within keloid disease itself, delineating specific features found in each category based on morphology, enabling easier diagnosis and management.

Basement membrane zone

The basement membrane zone (BMZ) at the dermo-epidermal junction, whilst described as thickened in KD with random discontinuities when compared to normal skin (Mogili et al., 2012), has not been comprehensively examined in the keloid research literature. The BMZ provides not only structural support but also crucially contributes to cell signalling, the regulation of cell trafficking and EMI (LeBleu et al., 2007; Breitkreutz et al., 2013; Bruckner-Tuderman and Has, 2014). One recent publication, using hyaluronan (HA) staining to classify scar types, describes the keloid BMZ as having shorter more cuboidal desmosomes when compared with normal skin and theorises that this may represent impaired epidermal barrier function (Hellstrom et al., 2014). There is very limited information on the expression of collagen IV (Ala-Kokko et al., 1987), collagen VII, perlecan, laminin, integrins and dystroglycans in relation to KD. With the recognition of the importance of EMI in wound healing and the likely demonstration of paracrine loops between keratinocytes and fibroblasts (Barton et al., 2010; Sobel et al., 2014), these BMZ components are likely to be altered in KD and may provide additional immunohistological and molecular markers for differentiating KD from other scarring entities.

Tissue microarray (TMA) and Next generation sequencing (NGS)

Originally referred to as the "sausage block" method, TMA allows high throughput screening, experimental uniformity and large sample number simultaneous analysis (Jawhar, 2009). Primarily used in tumour research the processing of multiple histological tissue sections under identical conditions is efficient and cost-effective (Kononen et al., 1998). Tissue-based assays including histochemistry, immunohistochemistry and in situ hybridisation can be performed on up to 1000 re-planted paraffin embedded core biopsies in a single block visualised on one slide. Application to heterogeneous tissue is not recommended, as the core biopsy may not be representative of the lesion as a whole (Barrette et al., 2014). Although keloid is heterogeneous it may be possible to use this technique to assess multiple areas of the same lesion at one time, similar to including multiple tumour progression stages on the one block. In this way, site-specific disease can be analysed and compared between keloid lesions and emerging patterns applied to differential diagnosis.

The advent of NGS (Hedegaard et al., 2014) may be of benefit to KD, especially as it has recently been shown to be applicable to formalin-fixed paraffin-embedded (FFPE) tissue sections (Corless and Spellman, 2012). While this technology is currently largely applied to oncology (Dander et al., 2014), the paucity of available fresh keloid tissue and potential numbers of archived FFPE samples that could be pooled, means NGS would be an ideal platform to apply to KD, also enabling comparison to other similar scarring entities (Sweeney and van de Rijn, 2012).

Summary and conclusions

KD is characterised clinically by patient and lesion heterogeneity resulting in inconsistent histological findings with mixed reports in the literature as well as varied response to therapy. The majority of recent focus in keloid has been on identifying genetic biomarkers to diagnose and target keloid scars, leaving histological descriptions incomplete.

Based on our literature search, we found the discerning features of keloid to be the epidermis and non-collagenous matrix molecules, altered by an imbalance in the phases of wound healing. The significant changes in these ECM molecules, attributed to prolonged proliferation and delayed remodelling phase, are summarised in Table 1 and the most common "pathognomonic" changes in Table 4. In some cases, it is the persistence of staining (decorin) or cellularity (myofibroblasts) that contributes to the interpretation rather than its definite presence or absence, highlighting the necessity of taking the age of the lesion into account. In addition to diagnostic value, these findings may help explain the aetiology of certain keloids. The evidence of reduced or occluded vascularity, particularly in the lesion centre, supports the hypoxic theory that has been put forward as a contributor to this disease. Similarly, the structure of keloid collagen has been described as tendon-like, suggesting it was thickened to deal with increased mechanical stress, another postulation for keloid aetiology (Bux and Madaree, 2012). Normally populated by a number of adnexae including pilosebaceous units and sweat glands, the observations of keloid dermis have demonstrated a scarcity of these structures (Tan et al., 2011). Indeed, studies have alluded to some specimens containing draining sinus tracts and/or inflamed ruptured hair follicles, suggesting a chronic inflammatory role in keloid pathogenesis (Lee et al., 2004).

The histology panels in Figs. 1-3, showing keloid and normal skin histochemistry stained in our own laboratory, depict many of the characteristic features associated with keloid disease. The thickened, flattened epidermis with associated hyperkeratosis (Fig. 1A), whorls of haphazard hyalinised collagen (Fig. 1C), hyper-cellularity (Fig. 1D) and horizontal fibrous bands (Fig. 1G,H) are shown. Fig. 2 compares keloid and normal skin using Herovici staining (Fitzgerald et al., 1996; Turner et al., 2013), where immature collagen III stains blue and mature collagen I stains red. There is a striking difference in the size of the sub-epidermal and

PD between keloid and normal skin. This increased distance in keloid is dominated by purplish staining, suggesting a mixture of types I and III collagen compared to the dominance of collagen I in the RD. This transition occurs much more quickly in the normal skin where it fades to a fine wavy regular type I collagen pattern. This difference in ratio of collagen I:III between the papillary and reticular dermis has been alluded to previously in reference to keloid (Syed et al., 2011).

Fig. 3 emphasises the significance of using a combination of features to aid diagnosis. The blurring of the papillary-reticular boundary (Fig. 3A) and evolution from fine collagen fibres sub-epidermally to coarser thickened fibres in the deeper dermis (Fig. 3B) are easily identified in samples with a complete profile of tissue present. Frequently the signs are more subtle, in that the epidermis is thickened but not necessarily flattened (Fig. 3C) or perhaps the collagen may not be the thickened coarser collagen expected of keloid but the associated cellularity and hyper-proliferative epidermis still support the diagnosis. Many of the samples show signs of inflammation, particularly sub-epidermally. Occasionally, the microscopic elements less routinely associated with keloid, including the presence of nodules (Fig. 3E) and a focal point of eruptive collagen (Fig. 3F), which depend on the area of the keloid biopsied and can lead to confusion with other entities. Any residual overlap with histological features of other cutaneous fibroses could potentially be laid to rest by closing the gap in knowledge with regard to BMZ features, unstudied ECM molecules and cellular confirmation. Further research into these histological components forms just part of the future work that should be undertaken to better understand KD. From the comparisons of keloid with hypertrophic scar to date, it is apparent that age-related findings play an important part in differential diagnosis with many of the similar findings between these two diverging with increasing age.

Approaching KD from a pathobiological perspective enables histological discrimination, improved differential diagnosis and correlation with molecular analysis. Amidst the continued search for a target biomarker, the histomorphology of keloid scars remains the mainstay of diagnosis. The inherent heterogeneity within fibrosis and limited availability of keloid samples has resulted in a widely variable and conflicting description of the morphology and tissue architecture. This review clarifies and emphasises the "pathognomonic" features that allow critical but undervalued distinction from other conditions and also highlights the gaps within the literature that may form the basis of future work. Improved differential diagnosis serves not only to prevent misdiagnosis of sinister disease but also allows targeting with appropriate therapy. While keloid therapeutic options are not the focus of this review and are discussed in detail elsewhere in the literature (Viera et al., 2012; Gold et al., 2014), we have highlighted a number of histological markers that may be of therapeutic interest. This is especially important in KD, where despite a plethora of available therapies, there is no one effective treatment.

References

Abdel-Meguid A.M., Weshahy A.H., Sayed D.S., Refaiy A.E. and Awad S.M. (2014). Intralesional vs. contact cryosurgery in treatment of keloids: a clinical and immunohistochemical study. Int. J. Dermatol. 54, 468-475.

Abdou A.G., Maraee A.H., Al-Bara A.M. and Diab W.M. (2011). Immunohistochemical expression of TGF-beta1 in keloids and hypertrophic scars. Am. J. Dermatopathol. 33, 84-91.

Abdou A.G., Maraee A.H. and Saif H.F. (2014). Immunohistochemical evaluation of COX-1 and COX-2 expression in keloid and hypertrophic scar. Am. J. Dermatopathol. 36, 311-317.

Abe R., Donnelly S.C., Peng T., Bucala R. and Metz C.N. (2001). Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. J. Immunol. 166, 7556-7562.

Abergel R.P., Pizzurro D., Meeker C.A., Lask G., Matsuoka L.Y., Minor R.R., Chu M.L. and Uitto J. (1985). Biochemical composition of the connective tissue in keloids and analysis of collagen metabolism in keloid fibroblast cultures. J. Invest. Dermatol. 84, 384-390.

Aiba S. and Tagami H. (1997). Inverse correlation between CD34 expression and proline-4-hydroxylase immunoreactivity on spindle cells noted in hypertrophic scars and keloids. J. Cutan. Pathol. 24, 65-69.

Aiba S., Tabata N., Ishii H., Ootani H. and Tagami H. (1992). Dermatofibrosarcoma protuberans is a unique fibrohistiocytic tumour expressing CD34. Br. J. Dermatol. 127, 79-84.

Ala-Kokko L., Rintala A. and Savolainen E.R. (1987). Collagen gene expression in keloids: analysis of collagen metabolism and type I, III, IV, and V procollagen mRNAs in keloid tissue and keloid fibroblast cultures. J. Invest. Dermatol. 89, 238-244.

Altman D.A., Nickoloff B.J. and Fivenson D.P. (1993). Differential expression of factor XIIIa and CD34 in cutaneous mesenchymal tumors. J. Cutan. Pathol. 20, 154-158.

Alves J.V., Matos D.M., Barreiros H.F. and Bartolo E.A. (2014). Variants of dermatofibroma--a histopathological study. Anais brasileiros de dermatologia. 89, 472-477.

Amadeu T., Braune A., Mandarim-de-Lacerda C., Porto L.C., Desmouliere A. and Costa A. (2003). Vascularization pattern in hypertrophic scars and keloids: a stereological analysis. Pathol. Res. Pract. 199, 469-473.

Amadeu T.P., Braune A.S., Porto L.C., Desmouliere A. and Costa A.M. (2004). Fibrillin-1 and elastin are differentially expressed in hypertrophic scars and keloids. Wound. Repair. Regen. 12, 169-174.

Ammendola M., Zuccala V., Patruno R., Russo E., Luposella M., Amorosi A., Vescio G., Sammarco G., Montemurro S., De Sarro G., Sacco R. and Ranieri G. (2013). Tryptase-positive mast cells and angiogenesis in keloids: a new possible post-surgical target for prevention. Updates Surg. 65, 53-57.

Arbi S., Eksteen E.C., Oberholzer H.M., Taute H. and Bester M.J. (2015). Premature collagen fibril formation, fibroblast-mast cell interactions and mast cell-mediated phagocytosis of collagen in keloids. Ultrastruct. Pathol. 39, 95-103.

Ashcroft K.J., Syed F. and Bayat A. (2013). Site-specific keloid fibroblasts alter the behaviour of normal skin and normal scar

fibroblasts through paracrine signalling. PLoS One. 8, e75600.

Atiyeh B.S., Costagliola M. and Hayek S.N. (2005). Keloid or hypertrophic scar: the controversy: review of the literature. Ann. Plast. Surg. 54, 676-680.

Babu M., Diegelmann R. and Oliver N. (1989). Fibronectin is overproduced by keloid fibroblasts during abnormal wound healing. Mol. Cell. Biol. 9, 1642-1650.

Bagabir R., Byers R.J., Chaudhry I.H., Muller W., Paus R. and Bayat A. (2012). Site-specific immunophenotyping of keloid disease demonstrates immune upregulation and the presence of lymphoid aggregates. Br. J. Dermatol. 167, 1053-1066.

Bakry O.A., Samaka R.M., Basha M.A., Tharwat A. and El Meadawy I. (2014). Hematopoietic stem cells: do they have a role in keloid pathogenesis? Ultrastruct. Pathol. 38, 55-65.

Barr R.J. and Stegman S.J. (1984). Delayed skin test reaction to injectable collagen implant (Zyderm). The histopathologic comparative study. J. Am. Acad. Dermatol. 10, 652-658.

Barr R.J., Young E.M. Jr. and King D.F. (1986). Non-polarizable collagen in dermatofibrosarcoma protuberans: a useful diagnostic aid. J. Cutan. Pathol. 13, 339-346.

Barrette K., van den Oord J.J. and Garmyn M. (2014). Tissue microarray. J. Invest. Dermatol. 134, e24.

Barton C.E., Johnson K.N., Mays D.M., Boehnke K., Shyr Y., Boukamp P. and Pietenpol J.A. (2010). Novel p63 target genes involved in paracrine signaling and keratinocyte differentiation. Cell Death Dis. 1, e74.

Barzilai A., Lyakhovitsky A., Horowitz A. and Trau H. (2003). Keloid-like scleroderma. Am. J. Dermatopath. 25, 327-330.

Baum C.L. and Arpey C.J. (2005). Normal cutaneous wound healing: clinical correlation with cellular and molecular events. Dermatol. Surg. 31, 674-686; discussion 686.

Beer W., Baldwin H.C., Goddard J.R., Gallagher P.J. and Wright D.H. (1998). Angiogenesis in pathological and surgical scars. Hum. Path. 29, 1273-1278.

Bellini A. and Mattoli S. (2007). The role of the fibrocyte, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab. Invest. 87, 858-870.

Bertheim U. and Hellstrom S. (1994). The distribution of hyaluronan in human skin and mature, hypertrophic and keloid scars. Br. J. Plast. Surg. 47, 483-489.

Blakaj A. and Bucala R. (2012). Fibrocytes in health and disease. Fibrogenesis Tissue Repair 5, S6.

Bloor B.K., Tidman N., Leigh I.M., Odell E., Dogan B., Wollina U., Ghali L. and Waseem A. (2003). Expression of keratin K2e in cutaneous and oral lesions: association with keratinocyte activation, proliferation, and keratinization. Am. J. Pathol. 162, 963-975.

Boyce S.T. (1994). Epidermis as a secretory tissue. J. Invest. Dermatol. 102, 8-10.

Boyce D.E., Ciampolini J., Ruge F., Murison M.S. and Harding K.G. (2001). Inflammatory-cell subpopulations in keloid scars. Br. J. Plast. Surg. 54, 511-516.

Bozoky B., Savchenko A., Guven H., Ponten F., Klein G. and Szekely L. (2014). Decreased decorin expression in the tumor microenvironment. Cancer Med. 3, 485-491.

Breitkreutz D., Koxholt I., Thiemann K. and 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.

Bruckner-Tuderman L. and Has C. (2014). Disorders of the cutaneous basement membrane zone--the paradigm of epidermolysis bullosa. Matrix Biol. 33, 29-34.

Bucala R. (2012). Review Series--Inflammation & fibrosis. Fibrocytes and fibrosis. QJM 105, 505-508.

Bucala R., Spiegel L.A., Chesney J., Hogan M. and Cerami A. (1994). Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol. Med. 1, 71-81.

Buechner S.A., Winkelmann R.K., Lautenschlager S., Gilli L. and Rufli T. (1993). Localized scleroderma associated with Borrelia burgdorferi infection. Clinical, histologic, and immunohistochemical observations. J. Am. Acad. Dermatol. 29 190-196.

Butler P.D., Longaker M.T. and Yang G.P. (2008). Current progress in keloid research and treatment. J. Am. Coll. Surg. 206, 731-741.

Butler P.D., Wang Z., Ly D.P., Longaker M.T., Koong A.C. and Yang G.P. (2011). Unfolded protein response regulation in keloid cells. J. Surg. Res. 167, 151-157.

Bux S. and Madaree A. (2010). Keloids show regional distribution of proliferative and degenerate connective tissue elements. Cells Tissues Organs 191, 213-234.

Bux S. and Madaree A. (2012). Involvement of upper torso stress amplification, tissue compression and distortion in the pathogenesis of keloids. Med. Hypoth. 78, 356-363.

Carlson M.A. and Longaker M.T. (2004). The fibroblast-populated collagen matrix as a model of wound healing: a review of the evidence. Wound Repair Regen. 12, 134-147.

Carrino D.A., Mesiano S., Barker N.M., Hurd W.W. and Caplan A.I. (2012). Proteoglycans of uterine fibroids and keloid scars: similarity in their proteoglycan composition. Biochem. J. 443, 361-368.

Catherino W.H., Leppert P.C., Stenmark M.H., Payson M., Potlog-Nahari C., Nieman L.K. and Segars J.H. (2004). Reduced dermatopontin expression is a molecular link between uterine leiomyomas and keloids. Genes Chromosom. Cancer 40, 204-217.

Chen J., Zhuo S., Jiang X., Zhu X., Zheng L., Xie S., Lin B. and Zeng H. (2011). Multiphoton microscopy study of the morphological and quantity changes of collagen and elastic fiber components in keloid disease. J. Biomed. Optics 16, 051305.

Chen J., Zeng B., Yao H. and Xu J. (2013). The effect of TLR4/7 on the TGF-beta-induced Smad signal transduction pathway in human keloid. Burns 39, 465-472.

Chen R., Zhang Z., Xue Z., Wang L., Fu M., Lu Y., Bai L., Zhang P. and Fan Z. (2015). Protein-protein interaction network of gene expression in the hydrocortisone-treated keloid. Int. J. Dermatol. 54, 549-554.

Chong Y., Park T.H., Seo S.W. and Chang C.H. (2015). Histomorphometric analysis of collagen architecture of auricular keloids in an asian population. Dermatol. Surg. 41, 415-422.

Chou W.C., Takeo M., Rabbani P., Hu H., Lee W., Chung Y.R., Carucci J., Overbeek P. and Ito M. (2013). Direct migration of follicular melanocyte stem cells to the epidermis after wounding or UVB irradiation is dependent on Mc1r signaling. Nat. Med. 19, 924-929.

Corless C.L. and Spellman P.T. (2012). Tackling formalin-fixed, paraffin-embedded tumor tissue with next-generation sequencing. Cancer. Discov. 2, 23-24.

Cosman B. and Wolff M. (1972). Acne keloidalis. Plast. Reconstr. Surg. 50(1), 25-30.

Crawford J., Nygard K., Gan B.S. and O'Gorman D.B. (2015). Periostin induces fibroblast proliferation and myofibroblast persistence in hypertrophic scarring. Exp. Dermatol. 24(2), 120-126.

Criscione V.D. and Weinstock M.A. (2007). Descriptive epidemiology of

dermatofibrosarcoma protuberans in the United States, 1973 to 2002. J. Am. Acad. Dermatol. 56(6), 968-973.

Curran T.A. and Ghahary A. (2013). Evidence of a role for fibrocyte and keratinocyte-like cells in the formation of hypertrophic scars. J. Burn Care Res. 34(2), 227-231.

Da Costa V., Wei R., Lim R., Sun C.H., Brown J.J. and Wong B.J. (2008). Nondestructive imaging of live human keloid and facial tissue using multiphoton microscopy. Arch. Facial Plast. Surg. 10, 38-43.

Dalkowski A., Schuppan D., Orfanos C.E. and Zouboulis C.C. (1999). Increased expression of tenascin C by keloids *in vivo* and *in vitro*. Br. J. Dermatol. 141, 50-56.

Dander A., Baldauf M., Sperk M., Pabinger S., Hiltpolt B. and Trajanoski Z. (2014). Personalized Oncology Suite: integrating next-generation sequencing data and whole-slide bioimages. BMC Bioinformat. 15, 306.

Darby I.A., Laverdet B., Bonte F. and Desmouliere A. (2014). Fibroblasts and myofibroblasts in wound healing. Clin. Cosmet. Investig. Dermatol. 7, 301-311.

De Pasquale R., Dinotta F., Scuderi L., Musumeci M.L. and Micali G. (2009). Dermatofibrosarcoma protuberans. Giornale italiano di dermatologia e venereologia. 144, 199-203.

Di Meglio P., Perera G.K. and Nestle F.O. (2011). The multitasking organ: recent insights into skin immune function. Immunity. 35, 857-869.

Ding J. and Tredget E.E. (2014). The role of chemokines in fibrotic wound healing. Adv. Wound. Care (in press).

Dong X., Zhang C., Ma S. and Wen H. (2014). Mast cell chymase in keloid induces profibrotic response via transforming growth factor-beta1/Smad activation in keloid fibroblasts. Int. J. Clin. Exp. Pathol. 7, 3596-3607.

Eckert R.L. (1989). Structure, function, and differentiation of the keratinocyte. Physiol. Rev. 69, 1316-1346.

Ehrlich H.P., Desmouliere A., Diegelmann R.F., Cohen I.K., Compton C.C., Garner W.L., Kapanci Y. and Gabbiani G. (1994). Morphological and immunochemical differences between keloid and hypertrophic scar. Am. J. Pathol. 145, 105-113.

Eyden B., Banerjee S.S., Shenjere P. and Fisher C. (2009). The myofibroblast and its tumours. J. Clin. Pathol. 62, 236-249.

Fitzgerald A.M., Kirkpatrick J.J., Foo I.T. and Naylor I.L. (1996). Human skin histology as demonstrated by Herovici's stain: a guide for the improvement of dermal substitutes for use with cultured keratinocytes? Burns 22, 200-202.

Fong E.P., Chye L.T. and Tan W.T. (1999). Keloids: time to dispel the myths? Plast. Reconstr. Surg. 104, 1199-1202.

Fradette J., Larouche D., Fugere C., Guignard R., Beauparlant A., Couture V., Caouette-Laberge L., Roy A. and Germain L. (2003). Normal human Merkel cells are present in epidermal cell populations isolated and cultured from glabrous and hairy skin sites. J. Invest. Dermatol. 120, 313-317.

Frenkel J.S. (2014). The role of hyaluronan in wound healing. Int. Wound J. 11, 159-163.

Funayama E., Chodon T., Oyama A. and 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.

Gao F.L., Jin R., Zhang L. and Zhang Y.G. (2013). The contribution of melanocytes to pathological scar formation during wound healing. Int. J. Clin. Exp. Med. 6, 609-613.

Garner W.L. (1998). Epidermal regulation of dermal fibroblast activity. Plast. Reconstr. Surg. 102, 135-139.

Gauglitz G.G., Korting H.C., Pavicic T., Ruzicka T. and Jeschke M.G. (2011). Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. Mol. Med. 17, 113-125.

Gold M.H., McGuire M., Mustoe T.A., Pusic A., Sachdev M., Waibel J. and Murcia C. (2014). Updated international clinical recommendations on scar management: Part 2—algorithms for scar prevention and treatment. Derm. Surg. 40, 825-831.

Guo S. and Dipietro L.A. (2010). Factors affecting wound healing. J. Dent. Res. 89, 219-229.

Gurtner G.C., Werner S., Barrandon Y. and Longaker M.T. (2008). Wound repair and regeneration. Nature 453, 314-321.

Haertel E., Werner S. and Schafer M. (2014). Transcriptional regulation of wound inflammation. Semin. Immunol. 26, 321-328.

Hahn J.M., Glaser K., McFarland K L., Aronow B.J., Boyce S.T. and Supp D.M. (2013). Keloid-derived keratinocytes exhibit an abnormal gene expression profile consistent with a distinct causal role in keloid pathology. Wound Repair Regen. 21, 530-544.

Halper J. and Kjaer M. (2014). Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. Adv. Exp. Med. Biol. 802, 31-47.

Har-Shai Y., Mettanes I., Zilberstein Y., Genin O., Spector I. and Pines M. (2011). Keloid histopathology after intralesional cryosurgery treatment. J. Eur. Acad. Dermatol. Venereol. 25, 1027-1036.

Har-Shai Y., Sabo E., Rohde E., Hyams M., Assaf C. and Zouboulis C.C. (2006). Intralesional cryosurgery enhances the involution of recalcitrant auricular keloids: a new clinical approach supported by experimental studies. Wound Repair Regen. 14, 18-27.

Hassel J.C., Roberg B., Kreuter A., Voigtlander V., Rammelsberg P. and Hassel A.J. (2007). Treatment of ear keloids by compression, using a modified oyster-splint technique. Dermatol. Surg. 33, 208-212.

Hedegaard J., Thorsen K., Lund M.K., Hein A.M., Hamilton-Dutoit S.J., Vang S., Nordentoft I., Birkenkamp-Demtroder K., Kruhoffer M., Hager H., Knudsen B., Andersen C.L., Sorensen K.D., Pedersen J.S., Orntoft T.F. and Dyrskjot L. (2014). Next-generation sequencing of RNA and DNA isolated from paired fresh-frozen and formalin-fixed paraffin-embedded samples of human cancer and normal tissue. PLoS One 9, e98187.

Hellstrom M., Hellstrom S., Engstrom-Laurent A. and Bertheim U. (2014). The structure of the basement membrane zone differs between keloids, hypertrophic scars and normal skin: A possible background to an impaired function. J. Plast. Reconstr. Aesthet. Surg. 67, 1564-1572.

Hinz B. (2007). Formation and function of the myofibroblast during tissue repair. J. Invest. Dermatol. 127, 526-537.

Hinz B., Phan S.H., Thannickal V.J., Prunotto M., Desmouliere A., Varga J., De Wever O., Mareel M. and Gabbiani G. (2012). Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am. J. Pathol. 180, 1340-1355.

Hochman B., Vilas Boas F.C., Mariano M. and Ferreiras L.M. (2005). Keloid heterograft in the hamster (*Mesocricetus auratus*) cheek pouch, Brazil. Acta Cirurgica Brasileira 20, 200-212.

Hu Z.C., Tang B., Guo D., Zhang J., Liang Y.Y., Ma D. and Zhu J.Y. (2014). Expression of insulin-like growth factor-1 receptor in keloid and hypertrophic scar. Clin. Exp. Dermatol. 39, 822-828.

Huang C., Akaishi S., Hyakusoku H. and Ogawa R. (2012). Are keloid and hypertrophic scar different forms of the same disorder? A

fibroproliferative skin disorder hypothesis based on keloid findings. Int. Wound. J. 11, 517-522.

Hunasgi S., Koneru A., Vanishree M. and Shamala R. (2013). Keloid: A case report and review of pathophysiology and differences between keloid and hypertrophic scars. J. Oral. Maxillofac. Pathol. 17, 116-120.

Hunzelmann N., Anders S., Sollberg S., Schonherr E. and Krieg T. (1996). Co-ordinate induction of collagen type I and biglycan expression in keloids. Br. J. Dermatol. 135, 394-399.

Ikeda M., Naitoh M., Kubota H., Ishiko T., Yoshikawa K., Yamawaki S., Kurokawa M., Utani A., Nakamura T., Nagata K. and Suzuki S. (2009). Elastic fiber assembly is disrupted by excessive accumulation of chondroitin sulfate in the human dermal fibrotic disease, keloid. Biochem. Biophys. Res. Commun. 390, 1221-1228.

Iqbal S.A., Syed F., McGrouther D.A., Paus R. and Bayat A. (2010). Differential distribution of haematopoietic and nonhaematopoietic progenitor cells in intralesional and extralesional keloid: do keloid scars provide a niche for nonhaematopoietic mesenchymal stem cells? Br. J. Dermatol. 162, 1377-1383.

Iqbal S.A., Sidgwick G.P. and Bayat A. (2012). Identification of fibrocytes from mesenchymal stem cells in keloid tissue: a potential source of abnormal fibroblasts in keloid scarring. Arch. Dermatol. Res. 304, 665-671.

Javad F. and Day P.J. (2012). Protein profiling of keloidal scar tissue. Arch. Dermatol. Res. 304, 533-540.

Jawhar N.M. (2009). Tissue microarray: A rapidly evolving diagnostic and research tool. Ann. Saudi Med. 29, 123-127.

Kadoya K., Amano S., Nishiyama T., Inomata S., Tsunenaga M., Kumagai N. and Matsuzaki K. (2015). Changes in fibrillin-1 expression, elastin expression and skin surface texture at sites of cultured epithelial autograft transplantation onto wounds from burn scar excision. Int. Wound. J. (in press).

Kamath N.V., Ormsby A., Bergfeld W.F. and House N.S. (2002). A light microscopic and immunohistochemical evaluation of scars. J. Cutan. Pathol. 29, 27-32.

Kanitakis J. (2002). Anatomy, histology and immunohistochemistry of normal human skin. Eur. J. Dermatol. 12, 390-399.

Kao H.K., Chen B., Murphy G.F., Li Q., Orgill D.P. and Guo L. (2011). Peripheral blood fibrocytes: enhancement of wound healing by cell proliferation, re-epithelialization, contraction, and angiogenesis. Ann. Surg. 254, 1066-1074.

Kauh Y.C., Rouda S., Mondragon G., Tokarek R., diLeonardo M., Tuan R.S. and Tan E.M. (1997). Major suppression of pro-alpha1(I) type I collagen gene expression in the dermis after keloid excision and immediate intrawound injection of triamcinolone acetonide. J. Am. Acad. Dermatol. 37, 586-589.

Kielty C.M. (2006). Elastic fibres in health and disease. Expert. Rev. Mol. Med. 8, 1-23.

Kim M., Cho K.H., Lee J.H., Chang M.S. and Cho S. (2012). Intratumoral mast cell number is negatively correlated with tumor size and mitosis in dermatofibrosarcoma protuberans. Exp. Dermatol. 21, 559-561.

Kischer C.W. (1992). The microvessels in hypertrophic scars, keloids and related lesions: a review. J. Submicrosc. Cytol. Pathol. 24, 281-296.

Kischer C.W. and Brody G.S. (1981). Structure of the collagen nodule from hypertrophic scars and keloids. Scan. Electron. Microsc. (Pt 3), 371-376.

Kischer C.W. and Hendrix M.J. (1983). Fibronectin (FN) in hypertrophic scars and keloids. Cell. Tissue Res. 231, 29-37.

Kischer C.W., Thies A.C. and Chvapil M. (1982). Perivascular myofibroblasts and microvascular occlusion in hypertrophic scars and keloids. Hum. Pathol. 13, 819-824.

Kishimoto J., Ehama R., Wu L., Jiang S., Jian N. and Burgeson R.E. (1999). Selective activation of the versican promoter by epithelial-mesenchymal interactions during hair follicle development. Proc. Natl. Acad. Sci. USA 96, 7336-7341.

Knaggs H.E., Layton A.M., Morris C., Wood E.J., Holland D.B. and Cunliffe W.J. (1994). Investigation of the expression of the extracellular matrix glycoproteins tenascin and fibronectin during acne vulgaris. Br. J. Dermatol. 130, 576-582.

Knapp T.R., Daniels R.J. and Kaplan E.N. (1977). Pathologic scar formation. Morphologic and biochemical correlates. Am. J. Pathol. 86, 47-70.

Kononen J., Bubendorf L., Kallionimeni A., Bärlund, M., Schraml P., Leighton S., Torhorst J., Mihatsch M.J., Sauter G. and Kallionimeni O.-P. (1998). Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nature Med. 4, 844-847.

Kose O. and Waseem A. (2008). Keloids and hypertrophic scars: are they two different sides of the same coin? Dermatol. Surg. 34, 336-346.

Krishnaswamy V.R. and Korrapati P.S. (2014). Role of dermatopontin in re-epithelialization: implications on keratinocyte migration and proliferation. Sci. Rep. 4, 7385.

Krishnaswamy V.R., Manikandan M., Munirajan A.K., Vijayaraghavan D. and Korrapati P.S. (2014). Expression and integrity of dermatopontin in chronic cutaneous wounds: a crucial factor in impaired wound healing. Cell. Tissue. Res. 358, 833-841.

Kuroda K., Okamoto O. and Shinkai H. (1999). Dermatopontin expression is decreased in hypertrophic scar and systemic sclerosis skin fibroblasts and is regulated by transforming growth factor-beta1, interleukin-4, and matrix collagen. J. Invest. Dermatol. 112, 706-710.

Kurokawa N., Ueda K. and Tsuji M. (2010). Study of microvascular structure in keloid and hypertrophic scars: density of microvessels and the efficacy of three-dimensional vascular imaging. J. Plast. Surg. Hand. Surg. 44, 272-277.

Lacour J.P., Vitetta A., Chiquet-Ehrismann R., Pisani A. and Ortonne J.P. (1992). Increased expression of tenascin in the dermis in scleroderma. Br. J. Dermatol. 127, 328-334.

Lavker R.M. and Schechter N.M. (1985). Cutaneous mast cell depletion results from topical corticosteroid usage. J. Immunol. 135, 2368-2373.

LeBleu V.S., Macdonald B. and Kalluri R. (2007). Structure and function of basement membranes. Exp. Biol. Med. 232, 1121-1129.

Lee J.Y., Yang C.C., Chao S.C. and Wong T.W. (2004). Histopathological differential diagnosis of keloid and hypertrophic scar. Am. J. Dermatopathol. 26, 379-384.

Lee W.J., Kim Y.O., Choi I.K., Rah D.K. and Yun C.O. (2011). Adenovirus-relaxin gene therapy for keloids: implication for reversing pathological fibrosis. Br. J. Dermatol. 165, 673-677.

Lee C.H., Hong C.H., Chen Y.T., Chen Y.C. and Shen M.R. (2012). TGF-beta1 increases cell rigidity by enhancing expression of smooth muscle actin: keloid-derived fibroblasts as a model for cellular mechanics. J. Dermatol. Sci. 67, 173-180.

Lee W.J., Choi I.K., Lee J.H., Kim Y.O. and Yun C.O. (2013). A novel three-dimensional model system for keloid study: Organotypic multicellular scar model. Wound Repair Regen. 21, 155-165.

Li-Tsang C.W., Feng B., Huang L., Liu X., Shu B., Chan Y.T. and

Cheung K.K. (2015). A histological study on the effect of pressure therapy on the activities of myofibroblasts and keratinocytes in hypertrophic scar tissues after burn. Burns. (in press).

Liang C.J., Yen Y.H., Hung L.Y., Wang S.H., Pu C. M., Chien H.F., Tsai J.S., Lee C.W., Yen F.L. and Chen Y.L. (2013). Thalidomide inhibits fibronectin production in TGF-beta1-treated normal and keloid fibroblasts via inhibition of the p38/Smad3 pathway. Biochem. Pharmacol. 85, 1594-1602.

Liang C.A., Jambusaria-Pahlajani A., Karia P.S., Elenitsas R., Zhang P.D. and Schmults C.D. (2014). A systematic review of outcome data for dermatofibrosarcoma protuberans with and without fibrosarcomatous change. J. Am. Acad. Dermatol. 71, 781-786.

Lim I.J., Phan T.T., Song C., Tan W.T. and 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.

Lim I.J., Phan T.T., Bay B.H., Qi R., Huynh H., Tan W.T., Lee S.T. and Longaker M.T. (2002). Fibroblasts cocultured with keloid keratinocytes: normal fibroblasts secrete collagen in a keloidlike manner. American journal of physiology. Cell. Physiol. 283, C212-222.

Linares H.A. and Larson D.L. (1974). Early differential diagnosis between hypertrophic and nonhypertrophic healing. J. Invest. Dermatol. 62, 514-516.

Lu F., Gao J., Ogawa R., Hyakusoku H. and Ou C. (2007). Biological differences between fibroblasts derived from peripheral and central areas of keloid tissues. Plast. Reconstr. Surg. 120, 625-630.

Luo L.F., Shi Y., Zhou Q., Xu S.Z. and Lei T.C. (2013). Insufficient expression of the melanocortin-1 receptor by human dermal fibroblasts contributes to excess collagen synthesis in keloid scars. Exp. Dermatol. 22, 764-766.

Luzar B. and Calonje E. (2010). Cutaneous fibrohistiocytic tumours - an update. Histopathology 56, 148-165.

Machesney M., Tidman N., Waseem A., Kirby L. and Leigh I. (1998). Activated keratinocytes in the epidermis of hypertrophic scars. Am. J. Pathol. 152, 1133-1141.

Martin C.W. and Muir I.F. (1990). The role of lymphocytes in wound healing. Br. J. Plast. Surg. 43, 655-662.

Martino M.M., Tortelli F., Mochizuki M., Traub S., Ben-David D., Kuhn G.A., Müller R., Livne E., Eming S.A. and Hubbell J.A. (2011). Engineering the growth factor microenvironment with fibronectin domains to promote wound and bone tissue healing. Sci. Translat. Med. 3, 100ra189-100ra189.

Massam-Wu T., Chiu M., Choudhury R., Chaudhry S.S., Baldwin A.K., McGovern A., Baldock C., Shuttleworth C.A. and Kielty C.M. (2010). Assembly of fibrillin microfibrils governs extracellular deposition of latent TGF beta. J. Cell. Sci. 123, 3006-3018.

Mathangi Ramakrishnan K., Meenakshi Janakiraman M. and Babu M. (2012). Expression of fibrocyte markers by keloid fibroblasts: an insight into fibrosis during burn wound healing - a preliminary study. Ann. Burns Fire Disasters 25, 148-151.

Matsuoka L.Y., Uitto J., Wortsman J., Abergel R.P. and Dietrich J. (1988). Ultrastructural characteristics of keloid fibroblasts. Am. J. Dermatopathol. 10, 505-508.

McCoy B.J., Diegelmann R.F. and Cohen I.K. (1980). *In vitro* inhibition of cell growth, collagen synthesis, and prolyl hydroxylase activity by triamcinolone acetonide. Exp. Biol. Med. 163, 216-222.

Merline R., Moreth K., Beckmann J., Nastase M.V., Zeng-Brouwers J., Tralhao J.G., Lemarchand P., Pfeilschifter J., Schaefer R.M., Iozzo R.V. and Schaefer L. (2011). Signaling by the matrix proteoglycan decorin controls inflammation and cancer through PDCD4 and MicroRNA-21. Sci. Signal. 4, ra75.

Meyer L.J., Russell S.B., Russell J.D., Trupin J.S., Egbert B.M., Shuster S. and Stern R. (2000). Reduced hyaluronan in keloid tissue and cultured keloid fibroblasts. J. Invest. Dermatol. 114, 953-959.

Mienaltowski M.J. and Birk D.E. (2014). Structure, physiology, and biochemistry of collagens. Adv. Exp. Med. Biol. 802 5-29.

Miragliotta V., Pirone A., Donadio E., Abramo F., Ricciardi M.P. and Theoret C.L. (2014). Osteopontin expression in healing wounds of horses and in human keloids. Equine Vet. J. (in press).

Mitts T.F., Bunda S., Wang Y. and Hinek A. (2010). Aldosterone and mineralocorticoid receptor antagonists modulate elastin and collagen deposition in human skin. J. Invest. Dermatol. 130, 2396-2406.

Mogili N.S., Krishnaswamy V.R., Jayaraman M., Rajaram R., Venkatraman A. and Korrapati P.S. (2012). Altered angiogenic balance in keloids: a key to therapeutic intervention. Translat. Res. 159, 182-189.

Moinzadeh P., Agarwal P., Bloch W., Orteu C., Hunzelmann N., Eckes B. and Krieg T. (2013). Systemic sclerosis with multiple nodules: characterization of the extracellular matrix. Arch. Dermatol. Res. 305, 645-652.

Moshref S.S. and Mufti S.T. (2010). Keloid and Hypertrophic scars: Comparative Histopathological and Immunohistochemical Study. J. King Abdulaziz Univ. Med. Sc. 17, 3-22.

Muller A.K., Meyer M. and Werner S. (2012). The roles of receptor tyrosine kinases and their ligands in the wound repair process. Semin. Cell Dev. Biol. 23, 963-970.

Mustoe T.A., Cooter R.D., Gold M.H., Hobbs F.D., Ramelet A.A., Shakespeare P.G., Stella M., Teot L., Wood F.M. and Ziegler U.E. (2002). International clinical recommendations on scar management. Plast. Reconst. Surg. 110, 560-571.

Naik-Mathuria B., Pilling D., Crawford J.R., Gay A.N., Smith C.W., Gomer R.H. and Olutoye O.O. (2008). Serum amyloid P inhibits dermal wound healing. Wound Repair. Regen. 16, 266-273.

Naitoh M., Hosokawa N., Kubota H., Tanaka T., Shirane H., Sawada M., Nishimura Y. and Nagata K. (2001). Upregulation of HSP47 and collagen type III in the dermal fibrotic disease, keloid. Biochem. Biophys. Res. Commun. 280, 1316-1322.

Namazi M.R., Fallahzadeh M.K. and Schwartz R.A. (2011). Strategies for prevention of scars: what can we learn from fetal skin? Int. J. Dermatol. 50, 85-93.

Natsuaki Y., Egawa G., Nakamizo S., Ono S., Hanakawa S., Okada T., Kusuba N., Otsuka A., Kitoh A., Honda T., Nakajima S., Tsuchiya S., Sugimoto Y., Ishii K.J., Tsutsui H., Yagita H., Iwakura, Y., Kubo M., Ng L., Hashimoto T., Fuentes J., Guttman-Yassky E., Miyachi Y. and Kabashima K. (2014). Perivascular leukocyte clusters are essential for efficient activation of effector T cells in the skin. Nat. Immunol. 15, 1064-1069.

Neill T., Schaefer L. and Iozzo R.V. (2012). Decorin: a guardian from the matrix. Am. J. Pathol. 181, 380-387.

Nie F.F., Wang Q. and Qin Z.L. (2008). The expression of fibrillin 1 in pathologic scars and its significance. Zhonghua Zheng Xing Wai Ke Za Zhi J. Plast. Surg. 24, 339-342. (in chinese).

Niessen F.B., Schalkwijk J., Vos H. and Timens W. (2004). Hypertrophic scar formation is associated with an increased number of epidermal Langerhans cells. J. Pathol. 202, 121-129.

Ogawa R., Watanabe A., Than Naing B., Sasaki M., Fujita A., Akaishi

S., Hyakusoku H. and Shimada T. (2014). Associations between keloid severity and single-nucleotide polymorphisms: importance of rs8032158 as a biomarker of keloid severity. J. Invest. Dermatol. 134, 2041-2043.

Ohyama M., Zheng Y., Paus R. and Stenn K.S. (2010). The mesenchymal component of hair follicle neogenesis: background, methods and molecular characterization. Exp. Dermatol. 19, 89-99.

Okamoto O., Fujiwara S., Abe M. and Sato Y. (1999). Dermatopontin interacts with transforming growth factor beta and enhances its biological activity. Biochem. J. 337, 537-541.

Olczyk P., Mencner L. and Komosinska-Vassev K. (2014). The role of the extracellular matrix components in cutaneous wound healing. Biomed. Res. Int. 2014, 747584.

Ong C.T., Khoo Y.T., Mukhopadhyay A., Masilamani J., Do D.V., Lim I.J. and Phan T.T. (2010). Comparative proteomic analysis between normal skin and keloid scar. Br. J. Dermatol. 162, 1302-1315.

Onodera M., Ueno M., Ito O., Suzuki S., Igawa H.H. and Sakamoto H. (2007). Factor XIIIa-positive dermal dendritic cells in keloids and hypertrophic and mature scars. Pathol. Int. 57, 337-342.

Pasparakis M., Haase I. and Nestle F.O. (2014). Mechanisms regulating skin immunity and inflammation. Nat. Rev. Immunol. 14, 289-301.

Paus R. (2013). Migrating melanocyte stem cells: masters of disaster? Nat. Med. 19, 818-819.

Peltonen J., Hsiao L.L., Jaakkola S., Sollberg S., Aumailley M., Timpl R., Chu M.L. and Uitto J. (1991). Activation of collagen gene expression in keloids: co-localization of type I and VI collagen and transforming growth factor-beta 1 mRNA. J. Invest. Dermatol. 97, 240-248.

Rakar J., Krammer M.P. and Kratz G. (2014). Human melanocytes mitigate keratinocyte-dependent contraction in an *in vitro* collagen contraction assay. Burns. (in press).

Ramirez F. and Rifkin D.B. (2009). Extracellular microfibrils: contextual platforms for TGFbeta and BMP signaling. Curr. Opin. Cell. Biol. 21, 616-622.

Reilkoff R.A., Bucala R. and Herzog E.L. (2011). Fibrocytes: emerging effector cells in chronic inflammation. Nat. Rev. Immunol. 11, 427-435.

Reinke J.M. and Sorg H. (2012). Wound repair and regeneration. Eur. Surg. Res. 49, 35-43.

Rencic A., Brinster N. and Nousari C.H. (2003). Keloid morphea and nodular scleroderma: two distinct clinical variants of scleroderma? J. Cutan. Med. Surg. 7, 20-24.

Russell S.B., Trupin J.S., Kennedy R.Z., Russell J.D. and 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.

Russell S.B., Russell J.D., Trupin K.M., Gayden A.E., Opalenik S.R., Nanney L.B., Broquist A.H., Raju L. and Williams S.M. (2010). Epigenetically altered wound healing in keloid fibroblasts. J. Invest. Dermatol. 130, 2489-2496.

Sabater-Marco V., Perez-Valles A., Berzal-Cantalejo F., Rodriguez-Serna M., Martinez-Diaz F. and Martorell-Cebollada M. (2006). Sclerosing dermatofibrosarcoma protuberans (DFSP): an unusual variant with focus on the histopathologic differential diagnosis. Int. J. Dermatol. 45, 59-62.

Santucci M., Borgognoni L., Reali U.M. and Gabbiani, G. (2001). Keloids and hypertrophic scars of Caucasians show distinctive morphologic and immunophenotypic profiles. Virchows Archiv. 438, 457-463.

Sarrazy V., Billet F., Micallef L., Coulomb B. and Desmouliere A. (2011). Mechanisms of pathological scarring: role of myofibroblasts and current developments. Wound Repair Regen. 19 (Suppl 1) s10-15.

Sayani K., Dodd C.M., Nedelec B., Shen Y.J., Ghahary A., Tredget E.E. and Scott P.G. (2000). Delayed appearance of decorin in healing burn scars. Histopathology 36, 262-272.

Schalkwijk J., Van Vlijmen I., Oosterling B., Perret C., Koopman R., Van den Born J. and Mackie E.J. (1991). Tenascin expression in hyperproliferative skin diseases. Br. J. Dermatol. 124, 13-20.

Schneider M., Meites E. and Daane S.P. (2013). Keloids: Which treatment is best for your patient? J. Fam. Prac. 62, 227-233.

Schnoor M., Cullen P., Lorkowski J., Stolle K., Robenek H., Troyer D., Rauterberg J. and Lorkowski S. (2008). Production of type VI collagen by human macrophages: a new dimension in macrophage functional heterogeneity. J. Immunol. 180, 5707-5719.

Scott P.G., Dodd C.M., Tredget E.E., Ghahary A. and Rahemtulla F. (1995). Immunohistochemical localization of the proteoglycans decorin, biglycan and versican and transforming growth factor-beta in human post-burn hypertrophic and mature scars. Histopathology 26, 423-431.

Seifert O. and Mrowietz U. (2009). Keloid scarring: bench and bedside. Arch. Dermatol. Res. 301, 259-272.

Seifert O., Bayat A., Geffers R., Dienus K., Buer J., Lofgren S. and Matussek A. (2008). Identification of unique gene expression patterns within different lesional sites of keloids. Wound Repair. Regen. 16, 254-265.

Shaker S.A., Ayuob N.N. and Hajrah N.H. (2011). Cell talk: a phenomenon observed in the keloid scar by immunohistochemical study. Appl. Immunohistochem. Mol. Morphol. 19, 153-159.

Shih B. and Bayat A. (2012). Comparative genomic hybridisation analysis of keloid tissue in Caucasians suggests possible involvement of HLA-DRB5 in disease pathogenesis. Arch. Dermatol. Res. 304, 241-249.

Shih B., Garside E., McGrouther D.A. and Bayat A. (2010). Molecular dissection of abnormal wound healing processes resulting in keloid disease. Wound Repair Regen. 18, 139-153.

Shrestha P., Sumitomo S., Lee C.H., Nagahara K., Kamegai A., Yamanaka T., Takeuchi H., Kusakabe M. and Mori M. (1996). Tenascin: growth and adhesion modulation--extracellular matrix degrading function: an *in vitro* study. Eur. J. Cancer. B. Oral. Oncol. 32B, 106-113.

Sible J.C., Eriksson E., Smith S.P. and Smith N. (1994). Fibronectin gene expression differs in normal and abnormal human wound healing. Wound Repair Regen. 2, 3-19.

Sidgwick G.P. and Bayat A. (2012). Extracellular matrix molecules implicated in hypertrophic and keloid scarring. J. Eur. Acad. Dermatol. Venerol. 26, 141-152.

Sidgwick G.P., Iqbal S.A. and Bayat A. (2013). Altered expression of hyaluronan synthase and hyaluronidase mRNA may affect hyaluronic acid distribution in keloid disease compared with normal skin. Exp. Dermatol. 22, 377-379.

Simone T.M., Longmate W.M., Law B.K. and Higgins P.J. (2014). Targeted inhibition of PAI-1 activity impairs epithelial migration and wound closure following cutaneous injury. Adv. Wound Care (in press).

Sobel K., Tham M., Stark H.J., Stammer H., Pratzel-Wunder S., Bickenbach J.R. and Boukamp P. (2014). Wnt-3a-activated human fibroblasts promote human keratinocyte proliferation and matrix destruction. International journal of cancer. J. Int. Cancer 136, 27,

86-98.

Soma T., Tajima M. and Kishimoto J. (2005). Hair cycle-specific expression of versican in human hair follicles. J. Dermatol. Sci. 39, 147-154.

Sorrell J.M. and Caplan A.I. (2009). Fibroblasts-a diverse population at the center of it all. Int. Rev. Cell. Mol. Biol. 276, 161-214.

Spiekman M., Przybyt E., Plantinga J.A., Gibbs S., van der Lei B. and Harmsen M.C. (2014). Adipose tissue-derived stromal cells inhibit TGF-beta1-induced differentiation of human dermal fibroblasts and keloid scar-derived fibroblasts in a paracrine fashion. Plast. Reconstr. Surg. 134, 699-712.

Suarez E., Syed F., Alonso-Rasgado T., Mandal, P. and Bayat, A. (2013). Up-regulation of tension-related proteins in keloids: knockdown of Hsp27, alpha2beta1-integrin, and PAI-2 shows convincing reduction of extracellular matrix production. Plast. Reconstr. Surg. 131, 158e-173e.

Sun L.M., Wang K.H. and Lee Y.C. (2014). Keloid incidence in Asian people and its comorbidity with other fibrosis-related diseases: a nationwide population-based study. Arch. Dermatol. Res. 306, 803-808.

Sundram U.N. (2009). Review: Dermatofibrosarcoma protuberans: histologic approach and updated treatment recommendations. Clin. Adv. Hematol. Oncol. 7, 406-408.

Supp D.M., Hahn J.M., Glaser K., McFarland K.L. and 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.

Sweeney R.T. and van de Rijn M. (2012). Microarrays and high-throughput sequencing in desmoid-type fibromatosis and scar. Desmoid Tumors 2012, 181-193.

Syed F., Ahmadi E., Iqbal S.A., Singh S., McGrouther D.A. and Bayat A. (2011). Fibroblasts from the growing margin of keloid scars produce higher levels of collagen I and III compared with intralesional and extralesional sites: clinical implications for lesional site-directed therapy. Br. J. Dermatol. 164, 83-96.

Tan E.M., Hoffren J., Rouda S., Greenbaum S., Fox J. 4th, Moore J.H. Jr and Dodge G.R. (1993). Decorin, versican, and biglycan gene expression by keloid and normal dermal fibroblasts: differential regulation by basic fibroblast growth factor. Exp. Cell Res. 209, 200-207.

Tan K.T., McGrouther D.A., Day A.J., Milner C.M. and Bayat A. (2011). Characterization of hyaluronan and TSG-6 in skin scarring: differential distribution in keloid scars, normal scars and unscarred skin. J. Eur. Acad. Dermatol. Venereol. 25, 317-327.

Teofoli P., Motoki K., Lotti T.M., Uitto J. and Mauviel A. (1997). Propiomelanocortin (POMC) gene expression by normal skin and keloid fibroblasts in culture: modulation by cytokines. Exp. Dermatol. 6, 111-115.

Theoret C.L., Olutoye O.O., Parnell L.K. and Hicks J. (2013). Equine exuberant granulation tissue and human keloids: a comparative histopathologic study. Vet. Surg. 42, 783-789.

Tholpady S.S., DeGeorge B.R. Jr, and Campbell C.A. (2014). The effect of local Rho-kinase inhibition on murine wound healing. Ann. Plast. Surg. 72, S213-S219.

Tirgan M.H., Shutty C.M. and Park T.H. (2013). Nine-month-old patient with bilateral earlobe keloids. Pediatrics 131, e313-317.

To W.S. and Midwood K.S. (2011). Plasma and cellular fibronectin: distinct and independent functions during tissue repair. Fibrogenesis Tissue Repair 4, 21.

Tracy L.E., Minasian R.A. and Caterson E. (2014). Extracellular matrix and dermal fibroblast function in the healing wound. Adv. Wound. Care (in press).

Trisliana Perdanasari A., Lazzeri D., Su W., Xi W., Zheng Z., Ke L., Min P., Feng S., Zhang Y.X. and Persichetti P. (2014). Recent developments in the use of intralesional injections keloid treatment. Arch. Plast. Surg. 41, 620-629.

Tsai Y.J., Lin P.Y., Chew K.Y. and Chiang Y.C. (2014). Dermatofibrosarcoma protuberans in children and adolescents: Clinical presentation, histology, treatment, and review of the literature. J. Plast. Reconstr. Aesthet. Surg. 67, 1222-1229.

Turner N.J., Pezzone M.A., Brown B.N. and Badylak S.F. (2013). Quantitative multispectral imaging of Herovici's polychrome for the assessment of collagen content and tissue remodelling. J. Tissue Eng. Regen. Med. 7, 139-148.

Ueda K., Furuya E., Yasuda Y., Oba S. and Tajima S. (1999). Keloids have continuous high metabolic activity. Plast. Reconstr. Surg. 104, 694-698.

Ueda K., Yasuda Y., Furuya E. and Oba S. (2004). Inadequate blood supply persists in keloids. Scand. J. Plast. Reconstr. Hand Surg. 38, 267-271.

Uitto J., Perejda A.J., Abergel R.P., Chu M.L. and Ramirez F. (1985). Altered steady-state ratio of type I/III procollagen mRNAs correlates with selectively increased type I procollagen biosynthesis in cultured keloid fibroblasts. Proc. Natl. Acad. Sci. USA 82, 5935-5939.

Van De Water, L., Varney, S. and Tomasek J.J. (2013). Mechanoregulation of the myofibroblast in wound contraction, scarring, and fibrosis: Opportunities for new therapeutic intervention. Adv. Wound. Care. 2, 122-141.

van den Broek L.J., Limandjaja G.C., Niessen F.B. and Gibbs S. (2014). Human hypertrophic and keloid scar models: principles, limitations and future challenges from a tissue engineering perspective. Exp. Dermatol. 23, 382-386.

Viera M.H., Vivas A.C. and Berman B. (2012). Update on keloid management: Clinical and basic science advances. Adv. Wound. Care. 1, 200-206.

Wang J., Hori K., Ding J., Huang Y., Kwan P., Ladak A. and Tredget E. E. (2011). Toll-like receptors expressed by dermal fibroblasts contribute to hypertrophic scarring. J. Cell. Physiol. 226, 1265-1273.

Wang Z., Liu X., Zhang D., Wang X., Zhao F., Shi P. and Pang X. (2015). Coculture with human fetal epidermal keratinocytes promotes proliferation and migration of human fetal and adult dermal fibroblasts. Mol. Med. Rep. 11, 1105-1110.

Weyrich A.S., Schwertz H., Kraiss L.W. and Zimmerman G.A. (2009). Protein synthesis by platelets: historical and new perspectives. J. Thromb. Haemost. 7, 241-246.

Xia W., Phan T.T., Lim I.J., Longaker M.T. and Yang G.P. (2006). Differential transcriptional responses of keloid and normal keratinocytes to serum stimulation. J. Surg. Res. 135, 156-163.

Yagi Y., Muroga E., Naitoh M., Isogai Z., Matsui S., Ikehara Y, Suzuki S., Miyachi Y. and Utani A. (2012). An ex vivo model employing keloid-derived cell-Seeded collagen sponges for therapy development. J. Invest. Dermatol. 133, 386-393.

Yagi Y., Muroga E., Naitoh M., Isogai Z., Matsui S., Ikehara S., Suzuki S., Miyachi Y. and Utani A. (2013). An ex vivo model employing keloid-derived cell-seeded collagen sponges for therapy development. J. Invest. Dermatol. 133, 386-393.

Young P., Fisher M., Segel J., Stucker F., Becker A. and Chen A.

(2013). Treatment of Large Keloids With Secondary-Intention Healing. Am. J. Cosmetic. Surg. 30, 193-199.

Zhang G., Ezura Y., Chervoneva I., Robinson P.S., Beason D.P., Carine E.T., Soslowsky L.J., Iozzo R.V. and Birk D.E. (2006). Decorin regulates assembly of collagen fibrils and acquisition of biomechanical properties during tendon development. J. Cell. Biochem. 98, 1436-1449.

Zhang Z., Nie F., Chen X., Qin Z., Kang C., Chen B., Ma J., Pan B. and Ma Y. (2015). Upregulated periostin promotes angiogenesis in keloids through activation of the ERK 1/2 and focal adhesion kinase pathways, as well as the upregulated expression of VEGF and angiopoietin-1. Mol. Med. Rep. 11, 857-864.

Zhou H.M., Wang J., Elliott C., Wen W., Hamilton D.W. and Conway, S.J. (2010). Spatiotemporal expression of periostin during skin development and incisional wound healing: lessons for human fibrotic scar formation. J. Cell. Commun. Signal. 4, 99-107.

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