



HAL
open science

The involvement of extracellular matrix proteins in the re-epithelialization of skin wounds

Patricia Rousselle, Marine Montmasson, Cécile Garnier

► To cite this version:

Patricia Rousselle, Marine Montmasson, Cécile Garnier. The involvement of extracellular matrix proteins in the re-epithelialization of skin wounds. *Matrix Biology*, 2019, 75-76, pp.12-26. <10.1016/j.matbio.2018.01.002>. <hal-03034642>

HAL Id: hal-03034642

<https://hal.science/hal-03034642v1>

Submitted on 1 Dec 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

The involvement of extracellular matrix proteins in the re-epithelialization of skin
wounds

Patricia Rousselle, Marine Montmasson and Cécile Garnier

Laboratoire de Biologie Tissulaire et Ingénierie Thérapeutique, UMR 5305, CNRS -
Université Lyon 1, Institut de Biologie et Chimie des Protéines, SFR BioSciences
Gerland-Lyon Sud, 7 passage du Vercors, F-69367, France.

Corresponding author

Patricia Rousselle: patricia.rousselle@ibcp.fr

List of abbreviations

Abbreviations: BM, basement membrane; BPA, Bullous Pemphigoid Antigen; DEJ, dermal-epidermal junction; ECM, extracellular matrix; EDA, extra domain-A; EGF, epidermal growth factor; FN, fibronectin; GAG, glycosaminoglycan; HSPG, heparan sulfate proteoglycan; LG, laminin domain; LM, laminin; MMPs, matrix metalloproteinases; OPN, osteopontin; RDEB, recessive dystrophic epidermolysis bullosa; 3D, three-dimensional; SPARC, secreted protein acidic and rich in cysteine; TGF, transforming growth factor; tPA, tissue-type plasminogen activator; TSP, thrombospondin.

Abstract

The ability of skin to act as a barrier is primarily determined by cells that maintain the continuity and integrity of skin and restore it after injury. Cutaneous wound healing in adult mammals is a complex multi-step process that involves overlapping stages of blood clot formation, inflammation, re-epithelialization, granulation tissue formation, neovascularization, and remodeling. Under favorable conditions, epidermal regeneration begins within hours after injury and takes several days until the epithelial surface is intact due to reorganization of the basement membrane. Regeneration relies on numerous signaling cues and on multiple cellular processes that take place both within the epidermis and in other participating tissues. A variety of modulators are involved, including growth factors, cytokines, matrix metalloproteinases, cellular receptors, and extracellular matrix components. Here we focus on the involvement of the extracellular matrix proteins that impact epidermal regeneration during wound healing.

Keywords: wound healing, re-epithelialization, epidermal regeneration, keratinocyte, extracellular matrix, basement membrane, laminin

Introduction

The ability of skin to act as a barrier is primarily determined by cells that maintain the continuity and integrity of skin and restore it after full-thickness wounds (Fig. 1A). Cutaneous wound healing is not, as was long believed, a simple linear process in which growth factors are synthesized that activate cell proliferation and migration. Rather, wound healing is a complex multi-step process that involves closely related dermal and epidermal events. Skin repair is the result of dynamic and interactive processes that involve soluble factors, blood elements, extracellular matrix (ECM) components, and cells [1]. In adult mammals, skin repair can be divided into four continuous phases, namely hemostasis, inflammation, proliferation (including re-epithelialization, granulation tissue formation, and neovascularization), and maturation or remodeling, and it generally leaves a scar [2, 3]. In mammals, blood clotting occurs first, and this provides chemotactic factors that attract inflammatory leukocytes and ECM proteins that serve as a substrate for migrating cells. Neutrophils help cleanse the wounded area and are eventually phagocytosed by macrophages, which enter the wound slightly later [4]. Macrophages also secrete growth factors that attract keratinocytes, fibroblasts, and blood vessels into the wound, thereby promoting re-epithelialization, granulation tissue formation, and vascularization [3, 5]. Both keratinocytes and fibroblasts synthesize key ECM components that are needed to re-form the basement membrane (BM) beneath the epidermal basal layer. There is wound contraction due to fibroblasts pulling on the ECM; this facilitates human wound remodeling, and the associated traction forces contribute to scarring [3]. The orchestration of these events relies on the spatial and temporal expression and the activation of a variety of proteins, such as growth factors, cytokines, matrix metalloproteinases (MMPs), and ECM components.

In contrast, wounds in mammalian embryos heal via rapid re-epithelialization and, in the absence of inflammation, via granulation tissue formation and contraction [6].

Epithelialization of wounds

Re-epithelialization is a critical step in the wound healing response (Fig. 1B). Defects in re-epithelialization lead to chronic non-healing wounds, such as venous stasis, diabetic and pressure ulcers [8]. Under favorable conditions, epidermal regeneration begins within hours after injury and continues until the epithelial surface is intact.

The inflammatory reaction to wounding results in the appearance of matrix molecules in the wound bed, with keratinocytes migrating rapidly from the edges of the wound and from hair follicles and sweat glands into the wound bed (Fig 1B) [11, 12, 13]. As fibroblasts begin to produce new ECM, epithelialization is initiated by epidermal growth factor (EGF) and transforming growth factor (TGF) α , which is produced by platelets, macrophages, and keratinocytes [14, 15, 16].

Normally, the epithelialization of wounds involves an orderly series of events in which keratinocytes migrate, proliferate, and differentiate to restore the barrier function [7]. Keratinocytes change shape and migrate, and this is one of the earliest and most crucial events in determining the efficiency of the overall wound repair process. Their migration is regulated at both the extracellular and intracellular levels and depends on the carefully balanced dynamic interaction of keratinocytes with ECM through adhesion complexes that form at matrix contact sites. These adhesion complexes involve transmembrane receptors such as integrins and syndecans. Migrating keratinocytes lose their apical-basal polarity, adhere to the provisional matrix, and project protrusive adhesion contacts such as filopodia and lamellipodia, which enables them to migrate laterally (Fig. 1B) [11]. Lateral migration requires profound reorganization of the

keratinocyte cytoskeleton, which loosens the adhesion complexes and intercellular junctions [17, 18, 19, 20]. MMP-9, which is produced by migrating keratinocytes, degrades BM components, allowing the keratinocytes to migrate over the wound [21]. Keratinocytes move into the healing wound by polymerizing cytoskeletal actin fibers in the outgrowth and by forming new adhesion complexes. Integrins and syndecans, which lack enzymatic activity, transmit intracellular signals by interacting with structural and signaling molecules. These events are accompanied by the expression of the following: integrin $\alpha\beta5$, $\alpha\beta6$, and $\alpha5\beta1$; various proteases, such as plasminogen and MMPs; growth factor receptors; cell surface proteoglycans; and ECM components like laminin (LM) 332 [22, 23, 24, 25]. This process proceeds until the migrating cells touch each other. The fusion of the opposing epithelia is accomplished by the degradation of the actin fibers in filopodia, as the actin fibers are replaced by intercellular adhesion contacts that finally close the wound much like a zipper [18]. When the epithelium has covered the wound bed, the proteins of the dermal-epidermal junction (DEJ) reappear in sequential order from the margins to the center of the wound, due to the concerted action of epidermal and dermal cells (Fig. 1A) [9, 10]. Basal keratinocytes then revert to a stationary phenotype in which they have apical polarity and are firmly anchored to the DEJ through reconstituted hemidesmosomes on their basal surface.

Epithelialization steps are stimulated by the local wound milieu, which typically shows altered ECM composition and the presence of cytokines that are produced by cells in the granulation tissue and by the clot [5]. In this review, we focus on the involvement of ECM proteins during the re-epithelialization phase of wound healing. Notably, the involvement of cytokines, growth factors, and MMPs has been described elsewhere [16, 26, 27].

The provisional ECM during epidermal repair

The migrating keratinocytes are in close contact with a provisional ECM that is associated with early wounds and is mainly composed of fibrin, plasma fibronectin (FN), vitronectin, and platelets [9, 28, 29]. There are two forms of FN: plasma FN, which is synthesized in a soluble form by hepatocytes into the blood plasma [30], and cellular or tissue FN, which is produced by other cells, such as fibroblasts, endothelial cells, and keratinocytes [31] (Fig. 2A). Plasma FN is more important in the early phase of wound healing when it binds to platelets and fibrin, thereby increasing the strength of the fibrin clot [30, 41, 49]. The strong adhesion of keratinocytes to the BM is disrupted when their hemidesmosomes dissolve; when their cell-cell connections are weakened, they then start to migrate underneath or through the temporary scaffolding that fills the wound defect [32]. In vivo studies have shown that plasma FN plays an important role in keratinocyte adhesion and migration [33]. Migrating keratinocytes are highly phagocytic, which allows them to penetrate through the clot. Degradation of the fibrin-FN clot appears to be critical for wound healing, since mice that lack the plasminogen gene or the uPA/tissue-type plasminogen activator (tPA) gene exhibit a number of wound abnormalities, including defects in wound healing and in keratinocyte migration [34, 35, 36].

Interestingly, keratinocytes migrate through tunnels of digested fibrin matrix in vitro, a process that requires plasminogen activation at the leading edge [37]. Tunnel formation and cell track length are differentially influenced by cytokines such as TGF β 1 and EGF in the wound fluid. The exact mechanisms underlying keratinocyte adherence to the fibrin matrix remain unknown, and it is also unknown whether this migration process takes place in vivo, where keratinocytes migrate collectively. The migration of

keratinocytes through a clot may be the result of the synergistic action of MMPs and plasmin.

FN plays a role in inflammation by promoting macrophage activation and opsonizing debris [9]. Keratinocytes deposit newly produced ECM proteins along their migration path, including cellular FN, tenascin C, and LM 332 (Fig. 3) [11, 38, 39, 40], suggesting that wound edge keratinocytes can modulate their own adhesion and migration substrates. Surprisingly, the wound healing process, including re-epithelialization, appears normal in plasma FN-deficient animals [53], suggesting that cellular FN may compensate for the lack of plasma FN. As the provisional matrix matures, cell-mediated remodeling takes place that is directed primarily by inflammatory cells and fibroblasts. This remodeling includes a transition from a fibrin-FN-dominated scaffold to a tissue-FN-dominated one that contains proteoglycans and interspersed crosslinked collagen; the tissue progressively becomes granulation tissue with which keratinocytes interact [41, 42].

Cellular fibronectin

FN is an important constituent of the vertebrate ECM that is ubiquitously expressed in human tissue and has long been known to be a major component in wound healing [43, Zollinger and Smith, 2017]. FN is multi-domain glycoprotein composed of an array of multiple repeated modular structures (Fig. 2A). It is secreted as a dimer maintained by two disulfide bonds at its C-terminus [30, 44]. The FN gene has three sites for alternative splicing: extra domain A (EDA) or extra type III homology A (EIIIA), EDB or EIIIB, and type III homologies connecting segment (IIICS; V region in rat) [Schwarzbauer, 1991]. Alternative splicing varies in a cell type-specific manner and

can modulate the functional properties of the molecule. In humans, over 20 main different isoforms are generated after mRNA processing, whereas in rats and mice, 12 variants are produced [French-Constant, 1995; Kornblihtt et al., 1996]. Alternative splicing of the FN gene is important in the context of wound repair [49, 131]. In situ hybridization studies on healing wounds in adult rat skin revealed that wounding induces FN mRNA expression both directly beneath the wounded area and in adjacent dermal tissue. This increased level of FN mRNA was evident within 1 d after wounding, with the highest levels seen at the fourth and seventh day. The splicing pattern of FN mRNA in the wound bed differs from that in normal skin. Cells at the base and edges of the wound expressed mRNA that included both of the alternatively spliced EDA and EDB regions; in contrast, mRNA expression in adjacent dermis and muscle showed the same splicing pattern as that seen in normal skin; that is without the spliced segments EDA and EDB. [49]. Macrophages were found as the first cells to express increased amounts of FN mRNA as early as 2 days after injury; some of these macrophage FN mRNAs contain the EDA and EDB domains [131, Gratchev et al., 2001]. Besides, at a late (7-day) interval after wounding, granulation tissue fibroblasts assumed the role of expressing FNs with and without the EDA and EDB fragments [131]. Additional studies revealed that, during their migration in human wounds, keratinocytes deposit EDA FN underneath the leading epithelial tongue, while both the EDA and EDB FN isoforms are found in the granulation tissue (Fig. 3) [48, 50]. In vitro, cultured keratinocytes leave behind trails of EDA FN that correlate with their migration [48]. Transgenic mice lacking the EDA exon in FN displayed major defects in re-epithelialization during the late phase of wound healing (day 5-7), consistent with the fact that in the first days after wounding, deposited FN is mainly derived from plasma [51]. In addition to the epidermal ulceration, the granulation tissue displayed

edematous regions causing abnormal healing of the wounds. Although no defects were seen in the healing of wounds in mice lacking the EDB segment, in vitro studies revealed reduced growth of fibroblasts and defaults in FN matrix assembly [52]. These data suggest that the EDA and EDB domains may have distinct functions.

The N-terminal portion of FN contains a self-assembly domain that allows individual FN dimers to come together in a process termed fibrillogenesis that results in the assembly of a three-dimensional (3D) matrix [45]. In contrast to collagen self-assembly, FN fibrillogenesis is a non-spontaneous cell-mediated process [46]. FN is secreted in a compact conformation and must be engaged by cell-surface integrins, most notably by $\alpha 5 \beta 1$ integrin, in order to reveal the cryptic self-association domains needed to form a definitive 3D matrix [47]. FN polymerization is a critical regulator of ECM organization and stability. It is essential for the deposition and retention of collagen-I and thrombospondin (TSP) 1 within the ECM and regulates the turnover and endocytosis of collagen I, which stabilizes collagen I matrix fibrils in the granulation tissue [54, 55]. Interestingly, a macrophages sub-population appears to participate in collagen endocytosis and degradation [Madsen et al., 2013].

Along with FN, the ECM is made up of collagens, proteoglycans, LMs, TSP 1 and tenascin.

Collagen

Dermal reconstitution begins approximately 3 to 5 days after injury, characterized by granulation tissue formation, which includes new blood vessel formation and the accumulation of fibroblasts. As dermal fibroblasts begin to migrate into the wound site, fibrillar collagen production gradually increases and the resulting collagen matrix will

ultimately replaces the provisional fibrin matrix [56]. Collagen constitutes more than 50% of the protein in scar tissue, and its production is essential to the healing process [Nimmi, 1974]. In the initial steps, collagen III and fibronectin are deposited and later on, collagen III is gradually replaced by collagen I of which deposition was observed at day 7 [57; Grinnell et al., 1981; Kurkinen et al., 1980; Mäkelä and Vuorio, 1986; Ignatz and Massague, 1986]. This was confirmed by in situ hybridization that revealed type I collagen mRNA in the upper layer of granulation tissue by days 6-13 after wounding [Scharffetter et al., 1989]. In early granulation tissue type III collagen is high (30%), whereas in the mature scar its expression goes back to that in intact dermis (10%) [Ehrlich and Krummel, 1996]. Despite that the migration tongue might have already significantly progressed before apparition of collagen I in granulation tissues, a number of studies have suggested a potential role for collagen I in re-epithelialization. A study reported that keratinocytes need to be activated by collagen I integrins before migration occurs [59], and another one revealed that keratinocytes that are in direct contact with collagen I begin to increase their production of collagenase [58]. Ligation of integrin $\alpha 2\beta 1$ to dermal matrix collagen I stimulates the expression and catalytic activity of MMP-1 in keratinocytes, thus facilitating directional keratinocyte migration over the dermal matrix [60]. MMP-1 is present in human cutaneous wounds during re-epithelialization but is no longer expressed when wound closure is complete [61]. Examination of healing wounds and a variety of chronic ulcers shows that MMP-1 is expressed by leading edge keratinocytes, but the surrounding epidermis shows decreased MMP-1 expression [61, 62]. The involvement of collagen I as a major substrate for keratinocyte migration during epithelialization was suggested by a study showing its capacity to promote keratinocyte migration in vitro [Woodley et al., 1988] and reinforced by findings reporting that fibrinogen, its derivatives, and the FN/fibrin

complex are anti-adhesive for keratinocytes. This study suggested that keratinocytes do not invade the fibrin-rich clot in vivo but rather migrate on a pathway between the fibrin clot and the collagen-rich viable dermis, using dermal collagen I as a substrate for their migration (Fig. 3) [9, 63]. Therefore additional studies are needed to elucidate whether collagen I impacts epidermal closure and how. Surprisingly, a recent study challenged the recognition of fibrillar collagens by collagen-binding integrins [Waltersdorf et al., 2017].

Collagen V was, in turn, proposed as implicated in the dermal wound healing response by modulating fibroblast behavior and tissue contraction (Berendsen et al., 2006). The classical Ehlers-Danlos syndrome, caused by collagen V gene mutations, displays skin fragility and abnormal wound healing (Martin, 2012). Additional studies in mice revealed that reduced collagen V expression (Wenstrup et al., 2006) is associated with decreased fibroblast proliferation, migration and reduced attachment to the ECM (De Nigris et al., 2016).

The presence of non-fibrillar collagen type VI has also been demonstrated in granulation tissue. It is deposited during the early stages of wound healing and its synthesis increases in parallel with type I collagen reaching a peak about 8 days post-wounding [Betz et al., 1993; Oono et al., 1993]. A recent study has demonstrated that type VI collagen is a key regulator of dermal matrix assembly and composition and that it influences fibroblasts behavior (Theocharidis et al., 2016). Interestingly, macrophages were shown to produce collagen VI to modulate their own binding properties (Schnoor et al., 2008).

Recent studies have revealed that other collagens may play roles in the repair process by directly or indirectly influencing the re-epithelialization process. An early analysis of

collagen VII expression did not support its potential involvement in skin epithelialization [11]; however, more recent studies of genetic recessive dystrophic epidermolysis bullosa (RDEB) disorders revealed that the loss of collagen VII results in delayed re-epithelialization by altering keratinocyte migration that is associated with the irregular deposition of LM 332. Moreover, the absence of collagen VII in skin delays fibroblast migration and granulation tissue formation [64]. These findings reinforce previous studies that showed that collagen VII plays a role in keratinocyte migration [65]. Besides, collagen VII was found in the provisional ECM under the epidermal tongue [64]. The topical application of recombinant collagen VII to full-thickness wounds in a murine wound healing model revealed that collagen VII stably incorporated into the DEJ and accelerated wound closure by increasing re-epithelialization and decreasing scarring and pro-fibrotic markers [66]. Applying collagen VII onto collagen VII-null skin grafted onto athymic nude mice corrected the poor dermal epidermal adherence and anchoring fibrils defects [66]. Collagen IV is not highly deposited in the provisional matrix under migrating keratinocytes in vivo. Immunohistochemical analysis of human skin wounds reveals its expression in BM fragments as early as four days after wounding. After 8 to 21 days, collagen IV is detected in newly formed BM [Betz et al., 1992; Larjava et al., 1993] confirming its role in BM assembly and stabilization.

Transmembrane collagen XVII, also known as the hemidesmosomal component Bullous Pemphogoid Antigen (BPA) 180, was found to play a role in keratinocyte adhesion to collagen IV and migration in vitro [67]. Further, its increased expression and the shedding of its collagenous ectodomain in acute skin wounds suggested that it might play a role in keratinocyte motility and proliferation [68]. Analysis of wound closure in mice that expressed a functional non-shedding collagen XVII revealed that collagen XVII ectodomain shedding is a highly dynamic modulator of in vivo

proliferation and motility in activated keratinocytes during epidermal regeneration [68, 69]. Further analysis revealed a role for collagen XVII in keratinocyte adhesion and directional motility through integrin-dependent PI3K activation and through stabilization of the lamellipodia at the leading edge of re-epithelializing wounds [70]. The heparan sulphate proteoglycan (HSPG) collagen XVIII is also suspected to play a role in wound healing. Genetically modified mice that lack collagen XVIII displayed accelerated wound healing [71]. In these mice, the vascularization rate of the wound bed was accelerated and the myofibroblast density was increased, suggesting that the accelerated healing was due to changes in wound contraction. Conversely, the healing of the wounds was delayed in mice overexpressing endostatin in skin, displaying abnormalities in epidermal and endothelial BMs but without significant effect on angiogenesis [71]. On the other hand, the use of endostatin in a rabbit ear hypertrophic scar model reduced the size of scars possibly through reduction of vascularization and angiogenesis [72].

Laminin

All LMs are composed of three different gene products, termed the α , β , and γ chains, that are assembled into an heterotrimer, $\alpha\beta\gamma$. LM α chains have a large LM globule (LG) at the carboxyl-terminal end that consists of five structurally similar domains (LG1 to LG5) that each contains about 200 residues (Fig. 2B) [73]. The major LM isoforms that are expressed in the DEJ, LM 332 (formerly called kalinin, epiligrin and nicein [73]), LM 511 and LM 521, appear to be involved in wound repair (Fig. 2B, C). Increased LM 332 expression is one of the earliest events in wound epithelialization, and LM 332 is expressed in epidermal keratinocytes within hours of injury. LM 332 appears to be the first BM component laid down onto the wound bed, as its expression precedes that of

all other ECM components (Fig. 3) [74, 75, 76, 77, 78]. In uninjured skin, LM 332 is synthesized by keratinocytes as a 460-kDa high molecular weight precursor protein. After secretion and deposition into the ECM, the $\alpha 3$ and $\gamma 2$ chains undergo maturation events consisting of loss of the 2 globular LG45 domains at the C-terminus of the $\alpha 3$ chain and loss of a 50-kDa fragment at the N-terminus of the $\gamma 2$ chain (Fig. 2B) [25]. These physiological post-translational processing events appear to allow the stable integration of LM 332 into the BM [Tunggal et al., 2002; Aumailley et al., 2003]. Mature LM 332 is the major component of anchoring filaments in skin [22] where it mediates cell adhesion via interaction of the $\alpha 3$ carboxyl-terminal LG1-3 triplet domain with both $\alpha 3\beta 1$ and $\alpha 6\beta 4$ integrins [23, 79, 80, 81], while the N-terminal short arms connect to BM components. In vivo, epidermal injury activates the transcription and deposition of LM 332 into the provisional matrix by the leading keratinocytes in the process of epidermal outgrowth and migration at the wound edge [74, 82, 83]. Precursor LM 332 is found in this provisional matrix, but it is absent from mature BMs [83,84]. Keratinocytes leave behind trails of precursor LM 332 while migrating on collagen I [77] or after stimulation by TGF β 1 [85]. Because LM 332 with a full-length $\alpha 3$ chain is found in epidermal repair [75, 86, 87], it was assumed that the LG45 domains also play a role. Indeed, a function for LG45 in the deposition of LM 332 in the ECM was hypothesized [25, 88], as was a direct function in keratinocyte adhesion and migration [87, 89, 90]. Syndecan-1 and -4 have been identified as cellular receptors for the LG45 domains [91, 92], supporting the idea that keratinocytes migrate by forming actin-based cellular protrusions [93] or by activating MMP-1 [91]. It has been shown that the expression and processing of the LG45 module is enhanced in keratinocytes after infection and in chronic wounds and that the level of expression and further processing correlate with the speed of wound healing [94]. Furthermore, LG45-derived peptides have

antimicrobial activity and chemotactic activity in mononuclear cells, suggesting that LG45 may also participate in host defense. The full-length $\gamma 2$ chain is also an important regulator of keratinocyte migration during wound repair [85, 95, 96], and it is hypothesized to be involved in LM 332 incorporation during BM assembly [97]. The $\alpha 3\beta 1$ integrin was identified as an important mediator of LM $\gamma 2$ processing, both in vivo during wound healing and in cultured keratinocytes (Longmate et al., 2014) through the upregulation of the mTLD/BMP-1 metalloproteases [98]. In contrast, the $\alpha 9\beta 1$ integrin plays a role in the inhibition of LM $\gamma 2$ processing by antagonizing $\alpha 3\beta 1$ -dependent mTLD/BMP-1 expression during re-epithelialization [98]. Studies of wound healing in conditional knockdown of the BMP-1/tolloid-like proteinases (BTPs) activity in mice, revealed delays in re-epithelialization and dramatic reduction in ECM deposition [Muir et al., 2016]. Incomplete processing of precursor ECM proteins, including that of the LM $\gamma 2$, is most likely involved in this mechanism [Muir et al., 2016].

LM 511 and LM 521 may also contribute to epidermal regeneration. Although they were described as potent keratinocyte adhesion substrates with the capacity to induce proliferation and migration in vitro [99], they were not found under migrating keratinocytes [102, Has and Nyström, 2015] and their expression and deposition in the maturing BM increase after re-epithelialization is complete [64, 102]. Their expression was found to correlate with the increased ability of keratinocytes to regenerate intact epithelium and detachment from LN-10/11 in the BM is likely to be a key factor in promoting keratinocyte differentiation [100]. Laminin 511 is an abundant isoform both at interfollicular sites and surrounding hair follicles that is synthesized by basal keratinocytes [Sorokin et al., 1997]. In a regenerative context, LM 511 was described as also produced by dermal pericytes located adjacent to the proliferative basal layer of the skin's epidermis [101]. Recent data from keratinocyte specific LM $\alpha 5$ KO suggest

that LM a5 is inhibitory of keratinocyte proliferation and migration through the control of epidermal and dermal growth factor expression [Wegner et al., 2016].

Perlecan

Perlecan has a structural bridging function and assemble the major constituents of the BM, collagen IV, LMs, and nidogen/entactin [Timpl and Brown, 1996; 103] within molecular suprastructures therefore creating molecular bridges that are compatible with cellular interactions [104]. Perlecan is the major proteoglycan component of BMs [Hassell et al., 1980; Paulsson et al., 1986] and as such acts as the reservoir for heparin-binding growth factors limiting their diffusion to act on cells on either side of the BM [Iozzo et al., 2009]. Perlecan's degradation during wound healing or tissue remodeling may act to quickly introduce necessary mitogens and trophic factors to begin regenerative processes. It can also instruct cells through direct interaction (Iozzo et al., 2009). A study reported that the BM HSPG perlecan deposition in the ECM of confluent keratinocytes strengthens cell adhesion and inhibits keratinocyte motility [102]. Furthermore, perlecan co-localizes with LM 332 at the wound margin of full thickness wounds in mice and concentrates where BM matures to stabilize keratinocyte adhesion (Fig. 3). This finding suggests the proximity of these two components during BM remodeling [102, 104]. Wound healing studies in perlecan heparan sulfate (HS)-deficient revealed significantly delayed wound healing due to reduced granulation tissue formation and decreased vascular density without affecting epidermal closure (Zou et al., 2004). In line with its potential involvement of in the late phases of epidermal regeneration, it was shown to play a role in keratinocyte survival and terminal differentiation [Sher et al., 2006]. A recent study also revealed its potential

to influence keratin 15 expression in keratinocytes [105], suggesting that it may influence keratinocytes stemness during wound repair.

Nidogens

The BM isoforms nidogens 1 and 2 bind to several BM-associated proteins and have been described as connecting elements between the LM and collagen IV networks thereby facilitating BM formation and stabilization (Aumailley et al., 1993; Fox et al., 1991; Kohfeldt et al., 1998). The analysis of mice lacking both isoforms, that die shortly after birth from numerous tissues abnormalities related to defects in BM assembly (Bader et al., 2005), revealed that skin displayed an ultrastructurally normal BM (Mokkapati et al., 2008) demonstrating that in vivo some BMs can still form without nidogen. A role of non-structural nature was proposed for nidogen 1 in skin wound repair (Baranowsky et al., 2010). Wound healing studies in nidogen 1 deficient mice revealed no defects in re-epithelialization but deficiency in the newly formed epidermis that showed marked hyperproliferation and delayed differentiation with an altered deposition of LM containing γ 1 chain.

Matricellular proteins

Matricellular proteins are multi-domain extracellular proteins. They do not appear to play structural roles in tissues. Rather, they are involved in a wide variety of cellular processes and are also able to influence cell behavior associated with wound healing. Many matricellular proteins show increased expression in response to injury, including TSP1 and TSP2, osteopontin (OPN), tenascin C, and SPARC [106].

Thrombospondin

TSP1 and TSP2 are matricellular proteins; as such, they are released and/or secreted following tissue injury and exert their cellular effects by associating with the ECM. While TSP1 and 2 are produced by fibroblasts, TSP1 is also a major component of platelet α -granules and is found in the circulation. TSP1 and TSP2 appear to play distinct, non-overlapping roles in the healing of skin wounds. Although TSP1 and TSP2 are dispensable for developmental angiogenesis, they are critical for proper tissue repair, both directly through their interactions with cell receptors and/or indirectly by modulation of the levels or bioavailability of effector molecules, including growth factors and MMPs [107]. In addition, TSP1 has been shown to be involved in the activation of latent TGF- β 1 [Murphy-Ullrich & Poczatek, 2000] and is considered as a major in vivo activator of TGF- β 1 as TSP1-null mice revealed suppressed active TGF- β 1 levels [Crawford et al., 1998]. The spatial and temporal expression of TSP1 and TSP2 are distinct in dermal wound healing [108, 109]. TSP1 is induced early in wound healing, concomitant with thrombus formation, edema, and inflammation. TSP2 expression occurs after resolution of the inflammatory phase and during granulation tissue remodeling. Wound healing studies in TSP1-null or knockdown mice indicate delayed repair that is characterized by loosely compacted and disorganized granulation tissue due to decreased levels of both total and active TGF β 1 and delayed re-epithelialization [108, 110]. Full thickness wounds in TSP2-null animals heal at an accelerated rate compared to WT mice and show alterations in the remodeling phase of healing [111]. Complete re-epithelialization of full thickness excisional wounds occurs earlier than in WT mice, and the epithelial layer is thicker. Further, granulation tissue at the wound site is disorganized: the morphology of collagen fibers is altered as is FN distribution;

these effects are most likely secondary to increased MMP-2 and MMP-9 expression [112].

Osteopontin

OPN is a phosphorylated acidic glycoprotein [113] that acts as a secreted chemokine-like protein and as part of an intracellular signaling complex, depending on the cellular context. A number of studies have reported elevated OPN expression in fibrosis, supporting the idea that OPN may contribute to inflammation-associated fibrosis. Even though OPN is needed for proper wound healing, it leads to fibrosis and excess scar formation if it persists for too long or in too high a concentration. OPN acts as a cytokine that is a chemoattractant for fibroblasts, and it appears to be necessary for fibroblast deposition of ECM components and collagen (Fig. 3) [114]. There are changes in the matrix architecture and in collagen fibril formation in the deep layers of wounds in OPN-deficient mice, indicating that OPN is involved in matrix reorganization [115]. One study reported faster re-epithelialization and decreased granulation tissue/scar formation in the healing of skin wounds of mice with acute local knockdown of OPN [116]. Macrophage-derived platelet derived growth factor (PDGF)-BB appears to be responsible for inflammation-induced OPN expression in wound fibroblasts, indicating that inflammation-triggered expression of OPN both hinders the rate of re-epithelialization and contributes to wound fibrosis.

Tenascin-C

Tenascin-C is a large hexameric extracellular glycoprotein. The founding member of a family of four tenascins, it is unique in its distinct pattern of expression. Little or no

tenascin-C is detected in healthy adult tissues. Tenascin-C has many extracellular binding partners, including matrix components, soluble factors; it also influences cell phenotype directly through interactions with cell surface receptors. Upon tissue damage, tenascin-C plays a multitude of different roles that mediate both inflammatory and fibrotic processes to enable effective tissue repair [Midwood and Orend, 2009].

Tenascin-C mRNA and protein expression is induced rapidly upon skin injury [117, 118, 119]. De novo expression of tenascin-C is a hallmark of inflammation, and a number of cytokines were shown to induce the gene (Chiquet-Ehrismann and Chiquet, 2003). It is expressed in areas of increased immune cell infiltration during the acute inflammation phase and it co-localizes with polymorphonuclear lymphocytes in the inflamed human dermis (Seyger et al., 1997). A role for tenascin-C in stimulating lymphocyte migration and activation was suggested (Clark et al. 1997, Nakahara et al. 2006). Tenascin-C was also shown to stimulate the production of pro-inflammatory cytokines TNF α , IL-6 and IL-8 in primary human macrophages and synovial fibroblasts, via activation of Toll-like receptor 4 mediated signaling pathways (Midwood et al. 2009). In addition its expression is also observed within 24 h of injury underneath migrating and proliferating keratinocytes [117, 120]. In situ hybridization demonstrates that keratinocytes are the major source of tenascin-C (Fig. 3) [121], suggesting that tenascin-C may help promote the proliferation and migration of epithelial cells. Tenascin-C is increased in the wound edges of the dermis associated with activated fibroblasts and extends throughout the dermis. High levels are also found in the new granulation tissue [117, 118, 120, 122], suggesting a role for tenascin-C in and promoting fibroblasts migration and tissue rebuilding during wound healing. Tenascin-C expression is not detected after wound repair is complete; indeed, tenascin-C levels return to normal after wound contraction, and the protein is not present in scar tissue

[117, 120, 122]. Its persistent expression is concomitant with chronic inflammation and it is persistently upregulated in fibrotic disease, including keloids and photo-damaged skin [Dalkowski et al, 1999; Filsel et al., 1999].

SPARC

SPARC (secreted protein acidic and rich in cysteine), also known as BM-40 and osteonectin, is a 32-kDa calcium-binding glycoprotein that is secreted by various cells, including fibroblasts, endothelial cells, and platelets [123]. Although SPARC is expressed in both the dermis and epidermis of uninjured skin, its expression increases during wound healing early after injury and reaches the highest level later during remodeling of the dermal ECM [124, 125]. Interestingly, SPARC-null mice display accelerated cutaneous wound closure [126], which could be due to the reduced collagen content of the SPARC-null dermis, which appears to render the skin intrinsically more susceptible to cell contraction. In addition to modulating the activity of a number of growth factors and ECM, SPARC may also influence MMP expression by several types of cells that participate in wound healing.

Proteoglycans and glycosaminoglycans (GAGs)

The involvement of proteoglycans and GAGs in wound healing is important as they play key roles in both fibrotic and regenerative wound healing. Their influence has mainly been described in the dermal repair processes, scar formation and fate [Tracy et al., 2016].

Final considerations

Several ECM proteins play roles in wound re-epithelialization, either via direct contact with keratinocytes or indirectly by affecting the granulation tissue. The quality of the granulation tissue, as well as its sequentially modified composition and structure, clearly influence the speed of epithelialization and the properties of the newly produced epidermis. While disorganized or excessive granulation tissue correlates with delayed re-epithelialization, decreased granulation tissue formation and/or enhanced contraction speeds re-epithelialization. In adult mammals, inflammatory cells such as macrophages start the process by providing growth factors, chemokines, cytokines and ECM proteins that activate the proliferation phase; this phase is beneficial and necessary for the timely repair of injured skin. Macrophages also source of ECM proteins and express high levels of proteases that can contribute to the cleavage and remodeling of ECM [Murray and Wynn, 2011]. Concomitantly, these early inflammatory signals appear to influence fibrosis and scar formation [27]. Interestingly, studies have revealed that wounds in the epidermis of zebrafish and mammalian embryos close via similar principles [6]. In these systems, wounds are re-epithelialized extremely rapidly and independently of inflammation and the ECM proteins supplied by the blood clot or granulation tissue [127]. This might be due to a predominant impact of tissue-autonomous extension movements within the re-epithelializing epidermis, a process that is likely to also occur in wounds of mammalian embryos (Caddy et al., 2010). In adult zebra fish, keratinocytes repopulate the wounds via TGF- β -dependent and integrin-dependent lamellipodial crawling at the leading edges of the epidermal tongue, thereby leading to epidermal closure that is independent of keratinocyte proliferation [128]. This process resembles primary healing in adult mammals in small or acute wounds where wound edges approximate. The actions of myofibroblasts are

minimized, and regeneration of the epidermis is optimal, since epidermal cells need to migrate only a minimal distance.

Future research will undoubtedly clarify which inflammatory cues and which ECM proteins (either produced by the keratinocytes themselves or present within the granulation tissue) are central to efficient re-epithelialization in adult mammals. This will identify putative targets for innovative therapeutic strategies that favor wound closure in clinical situations in which healing is delayed or prevented.

Acknowledgements

Work in the authors' laboratory was supported by the Agence Nationale de la Recherche (grant number ANR-13-RPIB-0003-01), by the Ligue Nationale Contre le Cancer and by CNRS in the framework of the GDR GAG (GDR 3739). MM and CG were both funded by the Agence Nationale de la Recherche (grant number ANR-13-RPIB-0003-01).

References

- [1] R.A.F. Clark, Wound repair. Lessons for tissue engineering, in: R.P. Lanza, R. Langer, W. Chick (Eds.), Principles of Tissue Engineering. Academic Press, San Diego, 1997, pp. 737–768.
- [2] P. Martin, Wound healing-aiming for perfect skin regeneration, *Science*. 276 (1997) 75–81.
- [3] T.J. Shaw, P. Martin, Wound repair at a glance. *J. Cell Sci.* 122 (2009) 3209–3213. doi:10.1242/jcs.031187
- [4] M.H. Kim, W. Liu, D.L. Borjesson, F.R. Curry, L.S. Miller, A.L. Cheung, et al., Dynamics of neutrophil infiltration during cutaneous wound healing and infection using fluorescence imaging, *J. Invest. Dermatol.* 128 (2008) 1812–1820. doi:10.1038/sj.jid.5701223
- [5] A.J. Singer, R.A.F. Clark, Cutaneous wound healing, *New Engl. J. Med.* 341 (1999) 738–746.
- [6] M.J. Redd, L. Cooper, W. Wood, B. Stramer, P. Martin, Wound healing and inflammation: embryos reveal the way to perfect repair, *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 359 (2004) 777–784. doi:10.1098/rstb.2004.1466
- [7] R. K. Sivamani, M.S. Garcia, R.R. Isseroff, Wound re-epithelialization: Modulating keratinocyte migration in wound healing, *Front. Biosci.* 12 (2007) 2849–2868. doi:10.2741/2277
- [8] N.S. Greaves, S.A. Iqbal, M. Baguneid, A. Bayat, The role of skin substitutes in the management of chronic cutaneous wounds. *Wound Repair Regen.* 21 (2013) 194–210. doi:10.1111/wrr.12029
- [9] R.A.F. Clark, Wound repair: overview and general considerations, in: R.A. Clark (Ed.), The molecular and cellular biology of wound repair (2nd ed.), Plenum Press, New-York, 1996, pp. 3–50.
- [10] D. Breitkreutz, I. Koxholt, K. Thiemann, R. Nischt, Skin basement membrane: the foundation of epidermal integrity-BM functions and diverse roles of bridging

- molecules nidogen and perlecan. *Biomed Res. Int.* E-pub. 2013 (2013) Article ID 179784, doi:10.1155/2013/179784.
- [11] H. Larjava, T. Salo, K. Haapasalmi, R.H. Kramer, J. Heino, Expression of integrins and basement membrane components by wound keratinocytes. *J. Clin. Invest.* 92 (1993) 1425–1435. doi:10.1172/JCI116719
- [12] I. Juhasz, G.F. Murphy, H.C. Yan, M. Herlyn, S.M. Albelda, Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing in vivo. *Am. J. Pathol.* 143 (1993) 1458–1469.
- [13] D.T. Woodley, Reepithelialization, in: R.A.F. Clark (Ed.), *The molecular and cellular biology of wound repair* (2nd ed.), Plenum Press, New-York, 1996, pp. 339–354.
- [14] R.A. Yates, L.B. Nanney, R.E. Gates, L.E. King, Epidermal growth factor and related growth factors, *Int. J. Dermatol.* 30 (1991) 687–694. doi:10.1111/j.1365-4362.1991.tb02609.x
- [15] G. Schultz, D.S. Rotatori, W. Clark, EGF and TGF- α in wound healing and repair, *J. Cell. Biochem.* 45 (1991) 346–352.
- [16] A. Michopoulou, P. Rousselle, How do epidermal matrix metalloproteinases support re-epithelialization during skin healing? *Eur. J. Dermatol.* 25 (2015) 33–42. doi: 10.1684/ejd.2015.2553
- [17] S.H. Litjens, J.M. de Pereda, A. Sonnenberg, Current insights into the formation and breakdown of hemidesmosomes. *Trends Cell. Biol.* 16 (2006) 376–383. doi:10.1016/j.tcb.2006.05.004
- [18] A. Jacinto, A. Martinez-Arias, P. Martin, Mechanisms of epithelial fusion and repair. *Nat. Cell Biol.* 3 (2001) 117–123. doi:10.1038/35074643
- [19] K. Stenn, L. Depalma, Re-epithelialization, in: *The Molecular and Cellular Biology of Wound Repair*, R.A.F. Clark, P. M. Henson (Eds.), Springer, Boston, 1988, pp. 321–335.

- [20] G. Odland, R. Ross, Human Wound Repair I. Epidermal Regeneration. *J. Cell Biol.* 39 (1968) 135-151.
- [21] G.J. Thomas, S. Poomsawat, M.P. Lewis, I.R. Hart, P.M. Speight, J.F. Marshall, Alpha v beta 6 integrin upregulates matrix metalloproteinase 9 and promotes migration of normal oral keratinocytes. *J. Invest. Dermatol.* 116 (2001) 898–904.
- [22] P. Rousselle, G.P. Lunstrum, D.R. Keene, R.E. Burgeson, Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J. Cell Biol.* 114 (1991) 567–576.
- [23] W.G. Carter, M.C. Ryan, P.J. Gahr, Epiligrin, a new cell adhesion ligand for integrin $\alpha 3\beta 1$ in epithelial basement membranes. *Cell* 65 (1991) 599–610.
- [24] K.J. Hamill, W.H. McLean, The alpha-3 polypeptide chain of laminin 5: Insight into wound healing responses from the study of genodermatoses. *Clin. Exp. Dermatol.* 30 (2005) 398-404. doi:10.1111/j.1365-2230.2005.01842.x
- [25] P. Rousselle, K. Beck, Laminin 332 processing impacts cellular behavior. *Cell Adh. Migr.* 7 (2013) 122–134. doi:10.4161/cam.23132
- [26] S. Werner, R. Grose, Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* 83 (2003) 835–870. doi:10.1152/physrev.00031.2002
- [27] J. Röhl, A. Zaharia, M. Rudolph, R.Z. Murray, The role of inflammation in cutaneous repair. *Wound Pract. Res.* 23 (2015) 8–15.
- [28] R.A.F. Clark, J.M. Lanigan, P. DellaPelle, E. Manseau, H.F. Dvorak, R.B. Colvin, Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization, *J. Invest. Dermatol.* 79 (1982) 264–269.
- [29] D. Chester, A.C. Brown, The role of biophysical properties of provisional matrix proteins in wound repair, *Matrix Biol.* 60–61 (2017) 124–140. doi:10.1016/j.matbio.2016.08.004
- [30] W.S To, K.S. Midwood, Plasma and cellular fibronectin: distinct and independent functions during tissue repair, *Fibrogenesis Tissue Repair.* (2011) 4:21. doi:10.1186/1755-1536-4-21

- [31] R.O. Hynes, K.M. Yamada, Fibronectins: multifunctional modular glycoproteins, *J. Cell Biol.* 95 (1982) 369–377. doi:10.1083/jcb.95.2.369
- [32] D.J. Donaldson, J.T. Mahan, Fibrinogen and fibronectin as substrates for epidermal cell migration during wound closure, *J. Cell Sci.* 62 (1983) 117–127.
- [33] K. Igisu, The role of fibronectin in the process of wound healing, *Thromb. Res.* 44 (1986) 455–465.
- [34] P. Carmeliet, L. Schoonjans, L. Kieckens, B. Ream, J. Degen, R. Bronson, R. De Vos, et al., Physiological consequences of loss of plasminogen activator gene function in mice, *Nature*, 368 (1994) 419–424.
- [35] T.H. Bugge, M.J. Flick, M.J.S. Danton, C.C. Daugherty, J. Rømer, K. Danø, P. et al. Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator, *Proc. Natl. Acad. Sci.* 93 (1996) 5899–5904.
- [36] J. Rømer, T.H. Bugge, C. Pyke, L.R. Lund, M.J. Flick, J.L. Degen, et al., Impaired wound healing in mice with a disrupted plasminogen gene, *Nat. Med.* 2 (1996) 287–292.
- [37] V. Ronfard, Y. Barrandon, Migration of keratinocytes through tunnels of digested fibrin, *Proc. Natl. Acad. Sci. U.S.A.* 98 (2001) 4504–4509. doi:10.1073/pnas.071631698
- [38] R.A.F. Clark, Fibronectin matrix deposition and fibronectin receptor expression in healing and normal skin. *J. Invest. Dermatol.* 94 (1990) 1285–1345.
- [39] A. Cavani, G. Zambruno, A. Marconi, V. Manca, M. Marchetti, A. Giannetti, Distinctive integrin expression in the newly forming epidermis during wound healing in humans. *J. Invest. Dermatol.* 101 (1993) 600–604.
- [40] J. Gailit, M.P. Welch, R.A.F. Clark, TGF-beta 1 stimulates expression of keratinocyte integrins during re-epithelialization of cutaneous wounds, *J. Invest. Dermatol.* 103 (1994) 221–227.
- [41] R.A.F. Clark, Fibronectin in the skin, *J. Invest. Dermatol.* 81 (1983) 475–479.

- [42] T.H. Barker, A.J. Engler, The provisional matrix: setting the stage for tissue repair outcomes, *Matrix Biol.* 60–61 (2017) 1–4. doi:10.1016/j.matbio.2017.04.003
- [43] F. Grinnell, R.E. Billingham, L. Burgess, Distribution of fibronectin during wound healing in vivo. *J. Invest. Dermatol.* 76 (1981) 181–189.
- [44] Y. Mao, J.E. Schwarzbauer, Stimulatory effects of a three-dimensional microenvironment on cell-mediated fibronectin fibrillogenesis. *J. Cell Sci.* 118 (2005) 4427–4436.
- [45] J.E. Schwarzbauer, Identification of the fibronectin sequences required for assembly of a fibrillar matrix. *J. Cell Biol.* 113 (1991) 1463–1473.
- [46] P. Singh, C. Carraher, J.E. Schwarzbauer, Assembly of fibronectin extracellular matrix. *Annu. Rev. Cell Dev. Biol.* 26 (2010) 397–419. doi:10.1146/annurev-cellbio-100109-104020
- [47] J.E. Schwarzbauer, J.L. Sechler, Fibronectin fibrillogenesis: a paradigm for extracellular matrix assembly. *Curr. Opin. Cell Biol.* 11 (1999) 622–627.
- [48] H. Larjava, L. Koivisto, L. Häkkinen, keratinocyte interactions with fibronectin during wound healing, in: *Madame Curie Bioscience Database [Internet]*. Austin (TX): Landes Bioscience; 2000-2013. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK6391/>
- [49] C. Ffrench-Constant, L. Van de Water, H.F. Dvorak, R.O. Hynes, Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat, *J. Cell Biol.* 109 (1989) 903–914.
- [50] P. Singh, C.L. Reimer, J.H. Peters, M.A. Stepp, R.O. Hynes, L. Van De Water, The spatial and temporal expression patterns of integrin alpha9beta1 and one of its ligands, the EIIIA segment of fibronectin, in cutaneous wound healing, *J. Invest. Dermatol.* 123 (2004) 1176-1181.
- [51] A.F. Muro, A.K. Chauhan, S. Gajovic, A. Iaconcig, F. Porro, G. Stanta et al., Regulated splicing of fibronectin EDA exon is essential for proper skin wound Healing and normal lifespan. *J. Cell Biol.* 162 (2003) 149–160.

- [52] T. Fukuda, N. Yoshida, Y. Kataoka, R. Manabe, Y. Mizuno-Horikawa, Sato M, et al., Mice lacking the edb segment of fibronectin develop normally but exhibit reduced cell growth and fibronectin matrix assembly in vitro. *Cancer Res.* 62 (2002) 5603–5610.
- [53] T. Sakai, K.J. Johnson, M. Murozono, K. Sakai, M.A. Magnuson, T. Wieloch et al., Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nat. Med.* 7 (2001) 324–330.
- [54] J. Sottile, D.C. Hocking, Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. *Mol. Biol Cell.* 13 (2002) 3546-3559.
- [55] F. Shi, J. Harman, K. Fujiwara, J. Sottile, Collagen I matrix turnover is regulated by fibronectin polymerization. *Am. J. Physiol. Cell Physiol.* 298 (2009) 1265–1275.
- [56] R. Raghow, The role of extracellular matrix in postinflammatory wound healing and fibrosis, *FASEB J.* 8 (1994) 823–831.
- [57] J.N. Clore, I.K. Cohen, R.F. Diegelmann, Quantitation of collagen types I and III during wound healing in rat skin. *Exp. Biol. Med.* 161 (1979) 337–340.
- [58] M.J. Petersen, D.T. Woodley, G.P. Stricklin, E.J. O'Keefe, Enhanced synthesis of collagenase by human keratinocytes cultured on type I or type IV collagen. *J. Invest. Dermatol.* 94 (1990) 341–346.
- [59] M. Guo, K. Toda, F. Grinnell, Activation of human keratinocyte migration on type I collagen and fibronectin. *J. Cell Sci.* 96 (1990) 197–205.
- [60] B.K. Pilcher, J.A. Dumin, B.D. Sudbeck, S.M. Krane, H.G. Welgus, W.C. Parks, The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. *J. Cell Biol.* 137 (1997) 1445-1457.
- [61] U.K. Saarialho-Kere, S.O. Kovacs, A.P. Pentland, J.E. Olerud, H.G. Welgus, W.C. Parks, Cell-matrix interactions modulate interstitial collagenase

- expression by human keratinocytes actively involved in wound healing. *J. Clin. Invest.* 92 (1993) 2858–2866.
- [62] U.K. Saarialho-Kere, M. Vaalamo, K. Airola, K.M. Niemi, A.I. Oikarinen, W.C. Parks, Interstitial collagenase is expressed by keratinocytes which are actively involved in re-epithelialization in blistering skin diseases. *J. Invest. Dermatol.* 104 (1995) 982–988.
- [63] M. Kubo, L. Van de Water, L.C. Plantefaber, M.W. Mosesson, M. Simon, M.G. Tonnesen, et al., Fibrinogen and fibrin are anti-adhesive for keratinocytes: a mechanism for fibrin eschar slough during wound repair. *J. Invest. Dermatol.* 117 (2001) 1369–1381.
- [64] A. Nyström, D. Velati, V.R. Mittapalli, A. Fritsch, J.S. Kern, L. Bruckner-Tuderman, Collagen VII plays a dual role in wound healing. *J. Clin. Invest.* 123 (2013) 3498–3509.
- [65] D.T. Woodley, Y. Hou, S. Martin, W. Li, M. Chen, Characterization of molecular mechanisms underlying mutations in dystrophic epidermolysis bullosa using site-directed mutagenesis. *J. Biol. Chem.* 283 (2008) 17838–17845.
- [66] X. Wang, P. Ghasri, M. Amir, B. Hwang, Y. Hou, M. Khalili, et al., Topical application of recombinant type VII collagen incorporates into the dermal-epidermal junction and promotes wound closure. *Mol. Ther.* 21 (2013) 1335–1344.
- [67] H. Qiao, A. Shibaki, H.A. Long, G. Wang, Q. Li, W. Nishie, et al., Collagen XVII participates in keratinocyte adhesion to collagen IV, and in p38MAPK-dependent migration and cell signaling. *J. Invest. Dermatol.* 129 (2009) 2288–2295.
- [68] J. Jacków, A. Schlosser, R. Sormunen, A. Nyström, C. Sitaru, K. Tasanen, et al., Generation of a functional non-shedding collagen xvii mouse model: relevance of collagen XVII shedding in wound healing. *J. Invest. Dermatol.* 136 (2016) 516–525.

- [69] J. Jacków, S. Löffek, A. Nyström, L. Bruckner-Tuderman, C.W. Franzke, Collagen XVII shedding suppresses re-epithelialization by directing keratinocyte migration and dampening mtor signaling. *J. Invest. Dermatol.* 136 (2016) 1031–1041.
- [70] S. Löffek, T. Hurskainen, J. Jacków, F.C. Sigloch, O. Schilling, K. Tasanen, et al., Transmembrane collagen XVII modulates integrin dependent keratinocyte migration via PI3K/Rac1 signaling. *PLoS One.* 9 (2014) e87263. doi:10.1371/journal.pone.0087263
- [71] L. Seppinen, R. Sormunen, Y. Soini, H. Elamaa, R. Heljasvaara, T. Pihlajaniemi, Lack of collagen XVIII accelerates cutaneous wound healing, while overexpression of its endostatin domain leads to delayed healing. *Matrix Biol.* 27 (2008) 535–546.
- [72] H.T. Ren, H. Hu, Y. Li, H.F. Jiang, X.L. Hu, C.M. Han. Endostatin inhibits hypertrophic scarring in a rabbit ear model. *J. Zhejiang Univ. Sci. B.* 14 (2013) 224–230.
- [73] M. Aumailley, L. Bruckner-Tuderman, W.G. Carter, R. Deutzmann, D. Edgar, P. Ekblom, et al., A simplified laminin nomenclature. *Matrix Biol.* 24 (2005) 326–332.
- [74] M.C. Ryan, R. Tizard, D.R. VanDevanter, W.G. Carter, Cloning of the LamA3 gene encoding the alpha 3 chain of the adhesive ligand epiligrin. Expression in wound repair. *J. Biol. Chem.* 269 (1994) 22779–22787.
- [75] M.C. Ryan, K. Lee, Y. Miyashita, W.G. Carter, Targeted disruption of the LAMA3 gene in mice reveals abnormalities in survival and late stage differentiation of epithelial cells. *J. Cell Biol.* 145 (1999) 1309–1323.
- [76] M.A. Kurpakus, E.L. Stock, J.C. Jones, Analysis of wound healing in an in vitro model: early appearance of laminin and a 125x10³ Mr polypeptide during adhesion complex formation. *J. Cell Sci.* 96 (1990) 651–660.

- [77] B.P. Nguyen, S.G. Gil, W.G. Carter, Deposition of laminin 5 by keratinocytes regulates integrin adhesion and signaling. *J. Biol. Chem.* 275 (2000) 31896–31907.
- [78] A.F. Laplante, L. Germain, F.A. Auger, V. Moulin, Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. *FASEB J.* 15 (2001) 2377–2389.
- [79] A. Sonnenberg, A.A. de Melker, A.M. Martinez de Velasco, H. Janssen, J. Calafat, C.M. Niessen, Formation of hemidesmosomes in cells of a transformed murine mammary tumor cell line and mechanisms involved in adherence of these cells to laminin and kalinin. *J. Cell Sci.* 106 (1993) 1083–1102.
- [80] P. Rousselle, M. Aumailley, Kalinin is more efficient than laminin in promoting adhesion of primary keratinocytes and some other epithelial cells and has a different requirement for integrin receptors. *J. Cell Biol.* 125 (1994) 205–214.
- [81] H. Mizushima, H. Takamura, Y. Miyagi, Y. Kikkawa, N. Yamanaka, H. Yasumitsu, et al., Identification of integrin-dependent and -independent cell adhesion domains in COOH-terminal globular region of laminin-5 α 3 chain. *Cell Growth Differ.* 8 (1997) 979–987.
- [82] T. Kainulainen, L. Hakkinen, S. Hamidi, K. Larjava, M. Kallioinen, J. Peltonen, et al., Laminin-5 expression is independent of the injury and the microenvironment during reepithelialization of wounds. *J. Histochem. Cytochem.* 46 (1998) 353–360.
- [83] P.D. Lampe, B.P. Nguyen, S. Gil, M. Usui, J. Olerud, Y. Takada, et al., Cellular interaction of integrin α 3 β 1 with laminin 5 promotes gap junctional communication, *J. Cell Biol.* 143 (1998) 1735–1747
- [84] L.E. Goldfinger, S.B. Hopkinson, G.W. deHart, S. Collawn, J.R. Couchman, J.C. Jones, The α 3 laminin subunit, α 6 β 4 and α 3 β 1 integrin coordinately regulate wound healing in cultured epithelial cells and in the skin. *J. Cell Sci.* 112 (1999) 2615–2629.

- [85] F. Decline, P. Rousselle, Keratinocyte migration requires $\alpha 2\beta 1$ integrin-mediated interaction with the laminin 5 $\gamma 2$ chain. *J. Cell Sci.* 114 (2001) 811–823.
- [86] E. Hintermann, V. Quaranta, Epithelial cell motility on laminin-5: regulation by matrix assembly, proteolysis, integrins and erbB receptors. *Matrix Biol.* 23 (2004) 75–85.
- [87] D.E. Frank, W.G. Carter, Laminin 5 deposition regulates keratinocyte polarization and persistent migration. *J. Cell Sci.* 117 (2004) 1351–1363.
- [88] R.O. Sigle, S.G. Gil, M. Bhattacharya, M.C. Ryan, T.M. Yang, T.A. Brown, et al., Globular domains 4/5 of the laminin $\alpha 3$ chain mediate deposition of precursor laminin 5, *J. Cell Sci.* 117 (2004) 4481–4494.
- [89] L.E. Goldfinger, M.S. Stack, J.C. Jones, Processing of laminin-5 and its functional consequences: role of plasmin and tissue-type plasminogen activator, *J. Cell Biol.* 141 (1998) 255–265.
- [90] O. Okamoto, S. Bachy, U. Odenthal, J. Bernaud, D. Rigal, H. Lortat-Jacob, et al., Normal human keratinocytes bind to the $\alpha 3$ LG4/5 domain of unprocessed laminin-5 through the receptor syndecan-1. *J. Biol. Chem.* 278 (2003) 44168–44177.
- [91] A. Utani, Y. Momota, H. Endo, Y. Kasuya, K. Beck, N. Suzuki, et al., Laminin $\alpha 3$ LG4 module induces matrix metalloproteinase-1 through mitogen-activated protein kinase signalling. *J. Biol. Chem.* 278 (2003) 34483–34490.
- [92] S. Carulli, K. Beck, G. Dayan, S. Boulesteix, H. Lortat-Jacob, P. Rousselle, Cell surface proteoglycans syndecan-1 and -4 bind overlapping but distinct sites in laminin $\alpha 3$ LG45 protein domain. *J. Biol. Chem.* 287 (2012) 12204–12216.
- [93] B. Sulka, H. Lortat-Jacob, R. Terreux, F. Letourneur, P. Rousselle, Tyrosine dephosphorylation of the syndecan-1 PDZ binding domain regulates syntenin-1 recruitment. *J. Biol. Chem.* 284 (2009) 10659–10671.
- [94] I. Senyürek, W.E. Kempf, G. Klein, A. Maurer, H. Kalbacher, L. Schäfer, et al., Processing of laminin α chains generates peptides involved in wound healing and host defense. *J. Innate. Immun.* 6 (2014) 467–484.

- [95] E. Natarajan, M. Saeb, C.P. Crum, S.B. Woo, P.H. McKee, J.G. Rheinwald. Co-expression of p16(INK4A) and laminin 5 gamma2 by microinvasive and superficial squamous cell carcinomas in vivo and by migrating wound and senescent keratinocytes in culture. *Am. J. Pathol.* 163 (2003) 477–491.
- [96] E. Natarajan, J.D. Omobono 2nd, Z. Guo, S. Hopkinson, A.J. Lazar, T. Brenn T, et al., A keratinocyte hypermotility/growth-arrest response involving laminin 5 and p16INK4a activated in wound healing and senescence. *Am J Pathol.* 168 (2006) 1821–1837.
- [97] L. Gagnoux-Palacios, M. Allegra, F. Spirito, O. Pommeret, C. Romero, J.P. Ortonne, et al., The short arm of the laminin gamma2 chain plays a pivotal role in the incorporation of laminin 5 into the extracellular matrix and in cell adhesion. *J. Cell Biol.* 153 (2001) 835–850.
- [98] W.M. Longmate, S.P. Lyons, L. DeFreest, L. Van De Water, C.M. DiPersio, Opposing roles of epidermal integrins $\alpha3\beta1$ and $\alpha9\beta1$ in regulation of mTLD/BMP-1-mediated laminin- $\gamma2$ processing during wound healing, *J. Invest. Dermatol.* (2017) 32945–32947. doi:10.1016/j.jid.2017.09.004
- [99] N. Pouliot, N.A. Saunders, P. Kaur, Laminin 10/11: an alternative adhesive ligand for epidermal keratinocytes with a functional role in promoting proliferation and migration, *Exp. Dermatol.* 11 (2002) 387–397.
- [100] A. Li, N. Pouliot, R. Redvers, P. Kaur, Extensive tissue-regenerative capacity of neonatal human keratinocyte stem cells and their progeny, *J. Clin. Invest.* 113 (2004) 390–400.
- [101] S. Paquet-Fifield, H. Schlüter, A. Li, T. Aitken, P. Gangatirkar, D. Blashk, et al., A role for pericytes as microenvironmental regulators of human skin tissue regeneration. *J. Clin. Invest.* 119 (2009) 2795–2806.
- [102] A. Botta, F. Delteil, A. Mettouchi, A. Viera, S. Estrach, L. Négroni, et al., Confluence switch signaling regulates ECM composition and plasmin proteolytic cascade in keratinocytes. *J. Cell Sci.* 125 (2012) 4341–4352.

- [103] D.T. Behrens, D. Villone, M. Koch, G. Brunner, L. Sorokin, H. Robenek, et al., The epidermal basement membrane is a composite of separate laminin- or collagen IV-containing networks connected by aggregated perlecan, but not by nidogens. *J. Biol. Chem.* 22 (2012) 18700–18709.
- [104] P.D. Yurchenco. Integrating activities of laminins that drive basement membrane assembly and function. *Curr. Top. Membr.* 76 (2015) 1–30.
- [105] M. Dos Santos, A. Michopoulou, V. André-Frei, S. Boulesteix, C. Guicher, G. Dayan, et al., Perlecan expression influences the keratin 15-positive cell population fate in the epidermis of aging skin, *Aging*, 8 (2016) 751–768.
- [106] P. Bornstein, E.H. Sage, Matricellular proteins: Extracellular modulators of cell function. *Curr. Opin. Cell Biol.* 14 (2002) 608–616.
- [107] T.R. Kyriakides, S. MacLauchlan, The role of thrombospondins in wound healing, ischemia, and the foreign body reaction. *J. Cell Commun. Signal.* 3 (2009) 215–225.
- [108] A. Agah, T.R. Kyriakides, J. Lawler, P. Bornstein, The lack of thrombospondin-1 (TSP1) dictates the course of wound healing in double-TSP1/TSP2-null mice. *Am. J. Pathol.* 161 (2002) 831–839.
- [109] T.R. Kyriakides, J.W. Tam, P. Bornstein, Accelerated wound healing in mice with a disruption of the thrombospondin 2 gene, *J. Invest. Dermatol.* 113 (1999) 782–787.
- [110] L. DiPietro, N. Nissen, R. Gamelli, A. Koch, J. Pyle, P. Polverini, Thrombospondin 1 synthesis and function in wound repair, *Am. J. Pathol.* 148 (1996) 1851–1860.
- [111] T.R. Kyriakides, Y.H. Zhu, Z. Yang, G. Huynh, P. Bornstein, Altered extracellular matrix remodeling and angiogenesis in sponge granulomas of thrombospondin 2-null mice, *Am. J. Pathol.* 159 (2001) 1255–1262.
- [112] S. MacLauchlan, E.A. Skokos, A. Agah, J. Zeng, W. Tian, J.M. Davidson et al., Enhanced angiogenesis and reduced contraction in thrombospondin-2-null wounds is associated with increased levels of matrix metalloproteinases-2 and -9, and soluble VEGF, *J. Histochem. Cytochem.* 57 (2009) 301–313.

- [113] D.R. Senger, D.F. Wirth, R.O. Hynes, Transformed mammalian cells secrete specific proteins and phosphoproteins, *Cell*, 16 (1979) 885–893.
- [114] F. Buback, A.C. Renkl, G. Schulz, J.M. Weiss. Osteopontin and the skin: multiple emerging roles in cutaneous biology and pathology, *Exp. Dermatol.* 18 (2009) 750–759.
- [115] L. Liaw, D.E. Birk, C.B. Ballas, J.S. Whitsitt, J.M. Davidson, B.L. Hogan. Altered wound healing in mice lacking a functional osteopontin gene (*spp1*), *J. Clin. Invest.* 101 (1998) 1468–1478.
- [116] R. Mori, T.J. Shaw, P. Martin, Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring, *J. Exp. Med.* 205 (2008) 43–51.
- [117] P. Betz, A. Nerlich, J. Tubel, R. Penning, W. Eisenmenger, Localization of tenascin in human skin wounds-an immunohistochemical study, *Int. J. Legal. Med.* 105 (1993) 325–328
- [118] M.A. Latijnhouwers, M. Bergers, B.H. Van Bergen, K.I. Spruijt, M.P. Andriessen, J. Schalkwijk, Tenascin expression during wound healing in human skin. *J. Pathol.* 178 (1996) 30–35.
- [119] K.S. Midwood, M. Chiquet, R.P. Tucker, G. Orend, Tenascin-C at a glance, *J. Cell Sci.* 129 (2016) 4321-4327.
- [120] E.J. Mackie, W. Halfter, D. Liverani, Induction of tenascin in healing wounds, *J. Cell Biol.* 107 (1998) 2757–2767.
- [121] M. Latijnhouwers, M. Bergers, M. Ponec, H. Dijkman, M. Andriessen, J. Schalkwijk, Human epidermal keratinocytes are a source of tenascin-C during wound healing, *J. Invest. Dermatol.* 108 (1997) 776–783.
- [122] R. Fassler, T. Sasaki, R. Timpl, M.L. Chu, S. Werner, Differential regulation of fibulin, tenascin-C, and nidogen expression during wound healing of normal and glucocorticoid-treated mice, *Exp. Cell Res.* 222 (1996) 111–116.

- [123] A.D. Bradshaw, E.H. Sage, SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury, *J. Clin Invest.* 107 (2001) 1049-1054.
- [124] M.J. Reed, P. Puolakkainen, T.F. Lane, D. Dickerson, P. Bornstein, E.H. Sage, Differential expression of SPARC and thrombospondin 1 in wound repair: immunolocalization and in situ hybridization, *J. Histochem. Cytochem.* 41 (1993) 1467–1477.
- [125] N. Hunzelman, M. Hafner, S. Anders, T. Krieg, R. Nischt, BM-40 (osteonectin, SPARC) is expressed both in the epidermal and in the dermal compartment of adult skin, *J. Invest. Dermatol.* 110 (1998) 122–126.
- [126] A.D. Bradshaw, M.J. Reed, E.H. Sage, SPARC-null mice exhibit accelerated cutaneous wound closure, *J. Histochem. Cytochem.* 50 (2002) 1–10.
- [127] R. Richardson, K. Slanchev, C. Kraus, P. Knyphausen, S. Eming, M. Hammerschmidt, Adult zebrafish as a model system for cutaneous wound-healing research, *J. Invest. Dermatol.* 133 (2013) 1655–1665.
- [128] R. Richardson, M. Metzger, P. Knyphausen, T. Ramezani, K. Slanchev, C. Kraus, et al., Re-epithelialization of cutaneous wounds in adult zebrafish combines mechanisms of wound closure in embryonic and adult mammals. *Development.* 143 (2016) 2077–2088.
- [129] R.O. Hynes, The dynamic dialogue between cells and matrices: Implications of fibronectin's elasticity, *Proc. Natl. Acad. Sci. U.S.A.* 96 (1999) 2588–2590.
- [130] J.E. Schwarzbauer, Alternative splicing of fibronectin: three variants, three functions. *Bioessays.* 13 (1991) 527–533.
- [131] L.F. Brown, D. Dubin, L. Lavigne, B. Logan, H.F. Dvorak, L. Van de Water, Macrophages and fibroblasts express embryonic fibronectins during cutaneous wound healing, *Am. J. Pathol.* 142 (1993) 793–801.

Figure legends

Fig. 1.

Diagram showing the re-epithelialization process. (A) Schematic drawing of the epidermis resting on the basement membrane (BM). Mammalian skin consists of the epidermis and dermis, which are separated by a BM. The epidermis is a stratified squamous epithelium that is composed of several cell layers, with the basal layer resting on the BM. The diagram shows adhesion structures, anchoring complexes, and BM organization. a, Laminin 332; b, Laminins 511/521; c, perlecan; d, collagen XVII; e, collagen VII; f, nidogen; g, collagen IV; h, syndecan; i, collagen I/III. (B) The re-epithelialization process 1 to 5 days post-wounding. Approximately 24 h after injury, activated basal and suprabasal keratinocytes adjacent to the wound edges undergo dramatic morphological changes. They acquire a motile phenotype and start migrating over the granulation tissue to cover the wounded area. Induction of a new set of genes leads to the expression of cellular receptors, growth factors, cytokines, matrix metalloproteinases and extracellular matrix proteins. Keratinocytes behind the wound edges begin proliferating and enrich the migrating tongue. The provisional matrix and granulation tissue are indicated.

Fig. 2.

Schematic representation showing human fibronectin (FN), laminin (LM) 332, LM 511 and LM 521. (A) FN is a multi-domain glycoprotein consisting of a series of 3 different modules, namely FNI, FNII, and FNIII repeats. These serve as building blocks for structural domains that have different functions [129]. For example, type I modules are found in fibrin binding domains, type II modules are found in collagen binding domains,

and type III modules are found in cell-binding domains [30]. Three alternatively spliced regions include the extra domain-A (EDA) and EDB regions, both type III repeats, and a variable (V) region [130]. Alternative splicing of the FN gene is important in the context of wound repair, since alternatively spliced forms are present in the matrix due to migrating keratinocytes as well as in the granulation tissue [48, 49, 131]. Insertion of additional type III repeats, EIIIA and EIIB, which flank the major cell adhesion domain of the molecule results in FNs EDA and EDB, respectively. EDA and EDB are present in so-called cellular FN but are absent from plasma FN. FN is secreted as a dimer in which the monomers are linked by disulfide bonds, which is essential for FN matrix assembly. (B) The structure of human LM 332 and its physiological maturation process. LM 332 is composed of three subunits: α 3A, β 3, and γ 2. Each chain has the indicated domains. The large LG structure located at the C-terminal end of the α 3 chain contains five repeating LG domains. The first three repeats, LG1–3, interact with the α 3 β 1, α 6 β 1, and α 6 β 4 integrins, while LG4 and LG5 contain binding sites for syndecan-1 and -4. LM 332 is synthesized as a precursor molecule that undergoes maturation by proteolytic processing at the α 3A chain N- and C-terminus as well as at the γ 2 chain N-terminus. The enzymes that are known to be involved are shown, and the cleavage sites are indicated by arrows. (C) Structure of human LMs 511 and 521.

Fig. 3.

Schematic showing the localization of extracellular matrix (ECM) components during re-epithelialization. The major ECM proteins are indicated in the vicinity of the epithelial tongue during epidermal regeneration 1 to 3 days post-injury (other extracellular molecules i.e. matrix metalloproteinases, cytokines, growth factors and ECM receptors are not represented here).

Figure 1

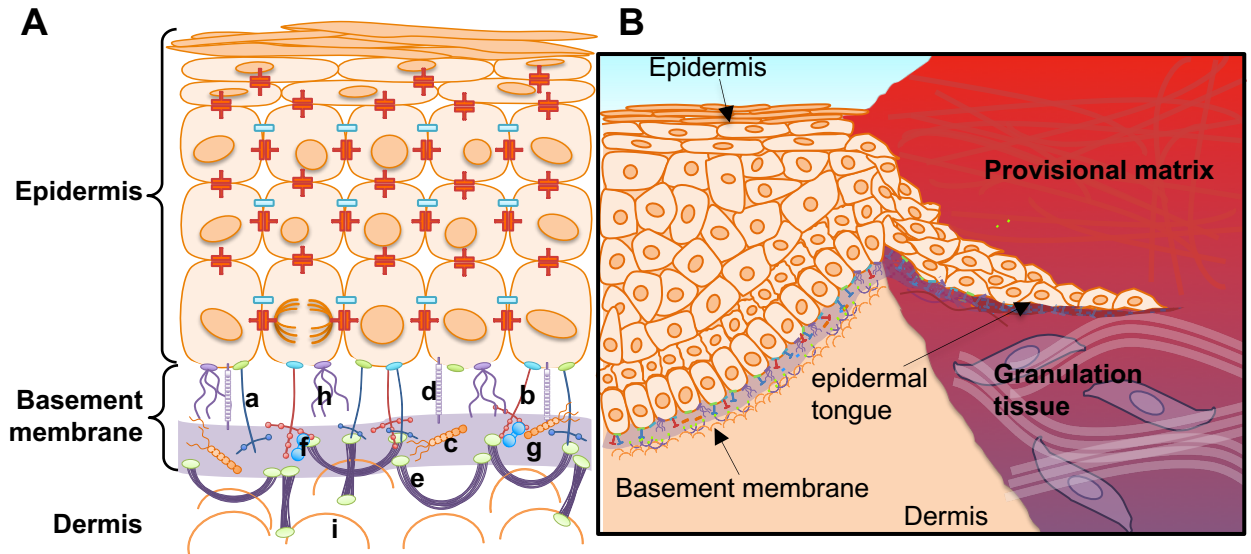


Figure 2

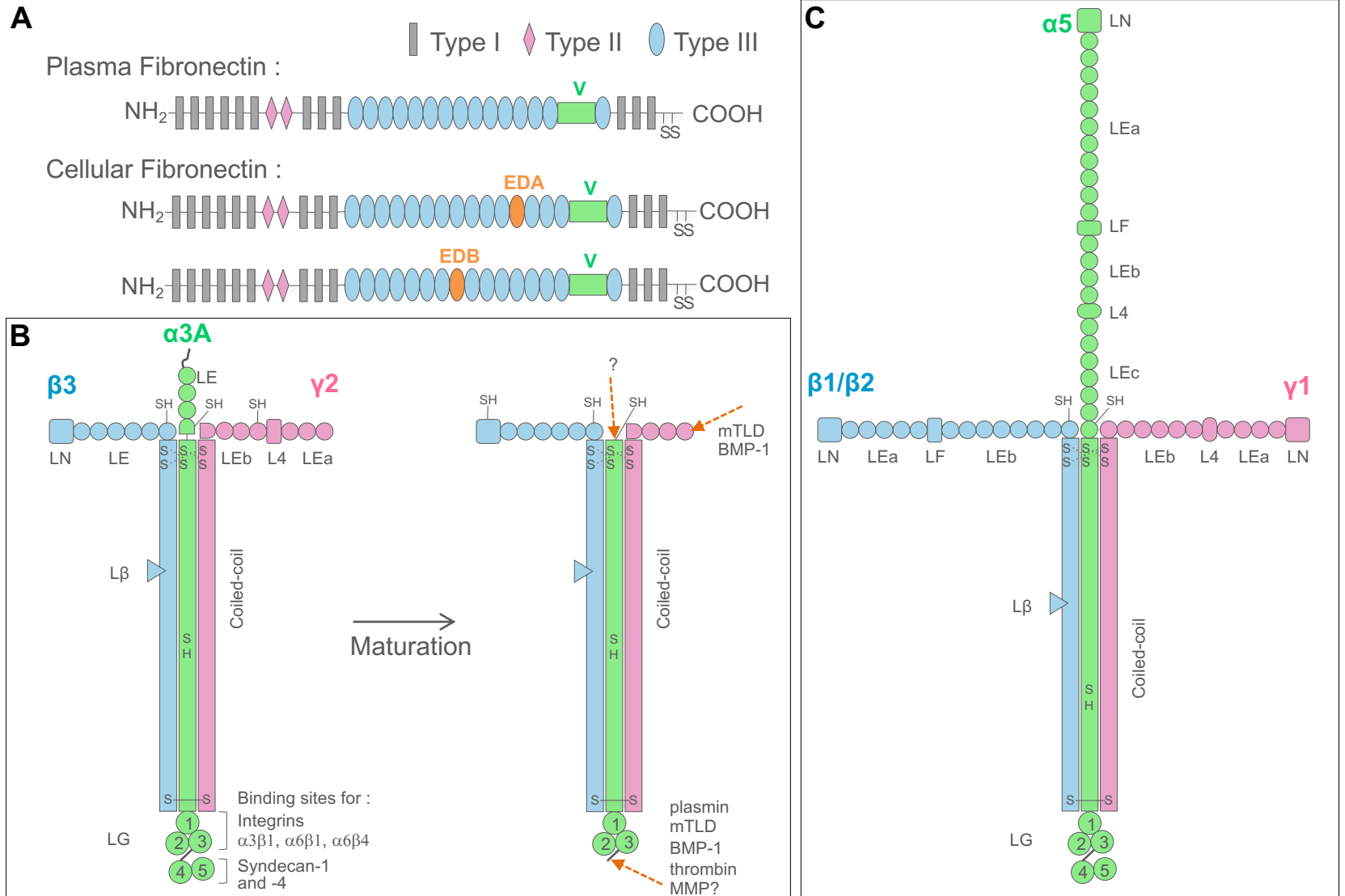


Figure 3

