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REVIEW

Fibronectin in tissue regeneration: timely disassembly of the scaffold is necessary to complete the build

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Abstract Tissue injury initiates extracellular matrix molecule expression, including fibronectin production by local cells and fibronectin leakage from plasma. To benefit tissue regeneration, fibronectin promotes opsonization of tissue debris, migration, proliferation, and contraction of cells involved in the healing process, as well as angiogenesis. When regeneration proceeds, the fibronectin matrix is fully degraded. However, in a diseased environment, fibronectin clearance is often disturbed, allowing structural variants to persist and contribute to disease progression and failure of regeneration. Here, we discuss first how fibronectin helps tissue regeneration, with a focus on normal cutaneous wound healing as an example of complete tissue recovery. Then, we continue to argue that, although the fibronectin matrix generated following cartilage and central nervous system white matter (myelin) injury initially benefits regeneration, fibronectin clearance is incomplete in chronic wounds (skin), osteoarthritis (cartilage), and multiple sclerosis (myelin). Fibronectin fragments or aggregates persist, which impair tissue regeneration. The similarities in fibronectin-mediated mechanisms of frustrated regeneration indicate that complete fibronectin clearance is a prerequisite for recovery in any tissue. Also, they provide common targets for developing therapeutic strategies in regenerative medicine.

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Fibronectin: elements of the scaffold

Fibronectin (Fn) is a high molecular weight glycoprotein that consists of three types of repeating amino acid units, named type I, type II, and type III repeats (Fig. 1). The structure of Fn depends on whether it is secreted in plasma or synthesized by resident cells. Plasma Fn (pFn) is produced by hepatocytes, and present in human blood at a concentration of 300 μg/ml [1, 2]. Cellular Fn (cFn) contains the alternatively spliced extra domain A (EDA) and/or extra domain B (EDB) (nomenclature for humans; for rodents: EIIIA and EIIIB). In addition, a third alternatively spliced domain, the IIICS domain (for rodents: the V-region), can be included, but regulations for its inclusion have not been fully discovered yet. pFn and cFn are secreted as a dimer, in which both subunits do not have to contain the same alternatively spliced variants. Physiological Fn monomers and dimers will hereafter be referred to as "native Fn". The main Fn receptors comprise a variety of integrin receptors (Fig. 1) [3]. In addition, Fn binds other extracellular matrix (ECM) molecules, including heparin, collagen, and fibrin, and together these protein networks form the ECM [4].

The main function of Fn is to serve as a scaffold for cell adhesion and migration, thereby also regulating cell proliferation and differentiation [4, 5]. These functions are supported by a variety of small proteins, such as growth factors, when they accumulate in the Fn network, increasing their concentration locally. Hence, these small molecules can be regarded as "the builders" on a scaffold of ECM including Fn, although Fn itself also stimulates tissue regeneration. The Fn matrix is essential for normal

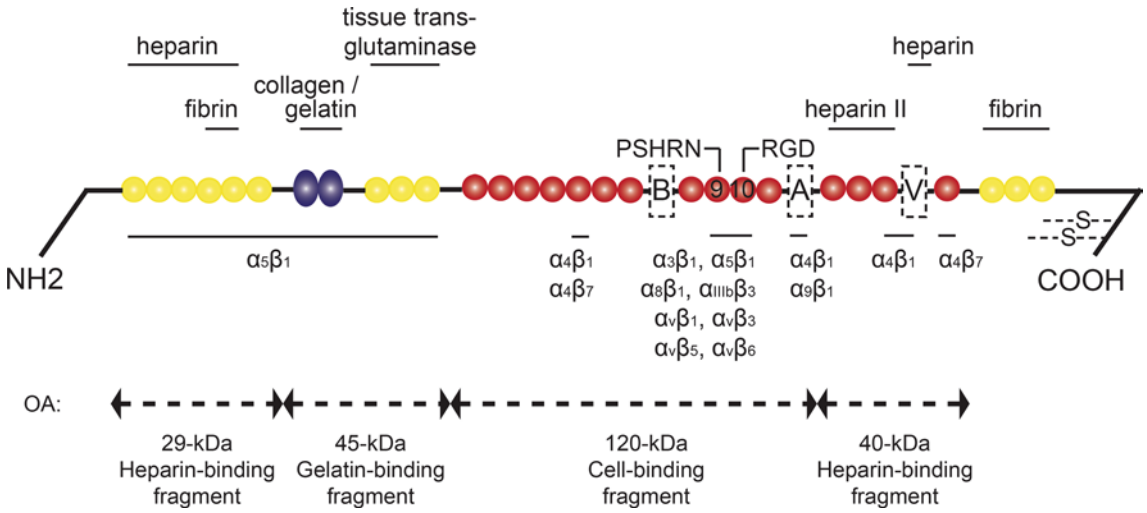

Fig. 1 Structure of fibronectin and major fibronectin fragments that are generated in osteoarthritis. The *yellow circles* represent type I, the *blue ellipses* type II, and the *red circles* type III repeats. The alternative splice variants are referred to as "A" for the EDA/EIIIA domain, "B" for the EDB/EIIIB domain, and V for the IIICS/V-region. Protein interaction sites are depicted above the linear structure, integrin binding sites below. PSHRN and RGD refer to these specific fibronectin domains. Four main fibronectin fragments with catabolic potential in osteoarthritis (OA) are shown under the *double-headed arrows*. The *arrows* correspond to the cleaving sites of these fragments. Adapted from [4, 48, 149]

embryonic development [6]. In healthy adult tissue, Fn is expressed at low levels. Transient Fn (re)-expression by plasma leakage and synthesis from resident cells is a common "default" response of tissue injury, ranging from skin wounds to joint inflammation [7] and myelin degradation (demyelination). Here, we discuss first how this temporary Fn matrix facilitates tissue regeneration, with a focus on normal cutaneous wound healing as an example of complete tissue regeneration. Next, we review how in osteoarthritis (OA) and multiple sclerosis (MS), clearance of the Fn matrix is disturbed, and contributes to failure of tissue regeneration via distinct mechanisms.

The fibronectin scaffold helps to rebuild tissue: focus on cutaneous wound healing

Wounds are defined as disruption of the normal anatomical structure and function of tissue. The wound-healing process is the physiological response to wounding in any tissue. Therefore, when we discuss how Fn benefits cutaneous wound healing, these functions equally apply to other tissues, although detailed features vary between tissue types [8]. In cutaneous wounds, regeneration involves (a) hemostasis and inflammation to provide temporary closure of the defect, (b) migration and proliferation of epithelial cells to replace the temporary seal, and (c) maturation and remodeling of the new epithelium and angiogenesis (reviewed, among many others, in [8–10]). Fn is involved in each of these steps to a greater or lesser extent (extensively reviewed in [11–13]).

On skin injury, a temporary Fn matrix ("the Fn scaffold") originates from plasma leakage and cellular expression. First, whole blood containing pFn leaks from the disrupted vessels and pFn is a major component of the subsequently formed hemostatic clot, although pFn is not essential for normal hemostasis [14]. Hemostatic thrombi are, however, more stable with pFn than without [15]. The hemostatic clot provides the basis of a provisional matrix, which also contains fibrin and other plasma proteins. The provisional matrix secures additional hemostasis, and assists migration and proliferation of epithelial cells. The presence of pFn in this matrix is not essential for normal wound healing [14], which is explained by compensatory actions of cFn. For as soon as a few hours after wounding [14], cells start to deposit Fn in the provisional matrix. Initially, mainly platelets secrete cFn [14], followed by macrophages, then fibroblasts [16], and possibly endothelial cells [17]. In addition, neutrophils express Fn mRNA at 24 h [17], but are negative again at 2 days after skin wounding [16]. Therefore, neutrophils also contribute to initial cFn expression early after wounding. Analogous to cutaneous wounds, a temporary Fn matrix is generated on cartilage damage [18] and myelin damage (demyelination) in the central nervous system (CNS) [19–23]. In these injuries, Fn leaks from plasma [19, 20] and is secreted by resident chondrocytes in cartilage [24, 25], and resident astrocytes, microglia, and endothelial cells in the CNS [23]. Therefore, the generation of a temporary Fn scaffold with pFn and cFn is a common response to tissue injury.

Functions attributed to the transient Fn matrix include stimulation of: (a) coating and ingestion (opsonization) from tissue debris by inflammatory cells, (b) migration and proliferation of regenerating cells via chemo- and/or haptotaxis, and (c) angiogenesis (for cutaneous wounds reviewed in: [9, 11, 13, 26]). Fn functions are similar among different tissue injuries, but mechanisms are best described in cutaneous wound healing. Fibroblast migration in wound healing requires functional RGD, heparin II, and cFn IIICS domains [27] (Fig. 1), and is promoted by EIIIA from cFn via β-catenin [28] and integrin α9β1 for keratinocytes [29]. Further, myofibroblast differentiation is stimulated by EIIIA [30] via integrin α4β7 [31]. In fact, EIIIA is essential for normal wound contraction in mice as shown via EIIIA knockout [32], although in mice from a different genetic background, EIIIA knockout did not impair wound healing [33]. Without EIIIB, mouse rib fractures heal normally, but more specific experiments are required to confirm its redundancy in cutaneous wound healing, especially since fibroblast proliferation and Fn matrix assembly in vitro are slightly reduced on EIIIB knockout [34].

Before tissue regeneration is completed, the Fn matrix is cleared. However, if Fn persists, this correlates to chronic failure of regeneration. In cutaneous wounds, analysis of wound fluid from human chronic venous ulcers showed persistence of Fn-degradation products, possibly as a result of increased matrix metalloproteinase 9 (MMP-9) activity [35, 36]. Certainly, failure of tissue regeneration is mediated by many factors, including changes in expression of growth factors, cytokines, and matrix proteins as well as receptor expression patterns, and tissue oxygen levels [37, 38]. Therefore, a structurally altered Fn matrix will only contribute to failure of regeneration in a complex interplay with changes in other factors, but nonetheless mediates tissue damage. Fn-degradation products in chronic venous ulcers, for example, likely stimulate neutrophil degranulation [39], and EIIIA activates Toll-like receptor 4 (TLR4) on inflammatory cells. This TLR4 stimulation may help opsonization of tissue debris at first, but eventually results in chronic inflammation [40, 41]. Interestingly, Fn fragments have also been implicated in OA disease progression, which will be discussed next.

Osteoarthritis: fibronectin fragments contribute to cartilage damage

OA is characterized by articular cartilage damage, resulting in joint destruction. The pathophysiology of OA is not fully understood. The current pathogenesis concept is based on increased cytokine and chemokine activation as a result of many factors, including ageing and chronic wear and tear on cartilage. In this concept, cytokines and chemokines contribute to protease production by chondrocytes, and inhibition of cartilage synthesis. This damages articular cartilage, and eventually also joint synovium, ligaments, tendons, and muscles [42, 43], causing pain and impairment of motility. The Fn matrix that is generated on the initial cartilage injury is not completely degraded in OA. As a result, Fn fragments contribute to (a) persistent local inflammation via innate immune system activation, and (b) direct cartilage damage.

Local joint inflammation results from Fn persistence in several ways. First, after Fn is degraded into multiple fragments by proteases (Fig. 1), Fn binds the C1q component of the complement system [44, 45], likely resulting in chronic stimulation of leukocytes. Secondly, the fragment containing EIIIA stimulates TLR4, as discussed above in the context of complicated wound healing [40, 46]. However, although the roles of EIIIA–TLR4 interactions in chronic wounds remain hypotheses based on in vitro studies, there is more evidence for the significance of this binding for joint damage. Injecting EIIIA-containing fragments into joints of mice results in joint swelling through the release of pro-inflammatory cytokines from mast cells [41]. Therefore, although innate immune system activation by Fn initially facilitates tissue debris clearance on cartilage damage, non-degraded Fn fragments contribute to chronic synovial inflammation in OA.

Cartilage damage in OA (and in rheumatoid arthritis) is also mediated by Fn fragments via suppression of sulfated proteoglycans, and via stimulation of chondrocytes and synovial fibroblasts to secrete catabolic cytokines and MMPs. The four major characterized Fn fragments include a 29-kDa heparin-binding fragment, a gelatin-binding fragment, a cell-binding fragment, and a 40-kDa heparin-binding fragment [47, 48] (Fig. 1). These Fn fragments are present at high levels in synovial fluid from OA patients [49], and in human osteoarthritic cartilage [50]. Fn fragments result from degradation of the Fn matrix, mediated by MMP-1, -3, -8, and -13 and by the aggrecanases ADAMTS-4 and -5 [48, 51, 52]. Native Fn has no adverse effects on cartilage, and low concentrations of Fn fragments show anabolic effects [53]. However, at higher concentrations, Fn fragments stimulate cartilage chondrolysis in vitro [54, 55] via different effector molecules and different pathways.

First, Fn fragments contribute to release of pro-inflammatory cytokines. These include IL-1, TNF-α, and IL-6 in cultured human cartilage for the 29-kDa heparin-binding fragment [56], and IL-6, IL-8 [57], and IL-7 [58] in human articular chondrocytes for the cell-binding fragment. These cytokines subsequently stimulate MMP expression from chondrocytes, including MMP-1, -2, -3, -9, and -13 [59–62], which enhance cartilage degradation. For example, chondrocytes release MMP-3 on stimulation with the

29-kDa heparin-binding fragment, but blocking antibodies to TNF-α, IL-1, and IL-6 suppress this release [56]. Activation of some of these cytokines and MMPs is mediated by the nuclear factor-κB (NF-κB) transcription pathway [57], whereas activation of others involves mitogen-activated protein kinase (MAPK) pathway activation (reviewed in [47]).

Secondly, Fn fragments induce MMP expression directly via cell surface receptors. For example, the 40-kDa heparin-binding fragment stimulates MMP release from chondrocytes via upregulation of NF-κB through the phosphoinositide-3-OH kinase (PI3K)/Akt pathway and the CD44 hyaluronan receptor [61, 63]. Interestingly, the same heparin-binding fragment also binds TLR4 to initiate aggrecanase release from chondrocytes [64]. The cell-binding Fn fragment binds integrin α5β1 on chondrocytes and fibroblasts, which induces the secretion of MMP-13 and degradation of cartilage [59, 65, 66]. This interaction requires reactive oxygen species as second messengers [67]. Although native Fn also binds to integrin receptors, native Fn stimulation does not cause a catabolic response from chondrocytes. This contrast can be explained by the hypothesis of "Fn-integrin imbalance". According to this hypothesis, Fn fragments alter normal Fn signals in chondrocytes by binding to distinct integrin receptors, but at the same time not binding to others. Therefore, chondrocytes perceive signals from altered clusters of integrins, and this initiates a catabolic response in OA [59, 68, 69]. Further, Fn fragments expose cryptic binding sites, which also explains the altered signaling compared to native Fn.

Thirdly, besides stimulation of cytokine and subsequent proteinase (MMP) production, Fn fragments, such as the 29-kDa heparin-binding fragment, damage cartilage via suppression of cartilage matrix synthesis, including sulfated proteoglycans [53, 70]. Also, the heparin-binding Fn fragment spanning the COOH-terminal induces an enhanced release of the free radical nitric oxide (NO) [71]. Although most of the data discussed are generated in vitro, additional in vivo studies show that injection of Fn fragments into rabbit knee joints results in cartilage destruction and joint swelling, resembling OA in humans [70, 72].

In order to reverse Fn fragment-mediated cartilage destruction in OA, and perhaps also rheumatoid arthritis, the following designs may be considered: (a) prevention of Fn fragmentation, (b) clearance of Fn fragments, and (c) by-pass of harmful Fn fragment signals. Attempts at by-passing Fn fragment signals is, to our knowledge, the only approach to have been tested so far. Anti-oxidants, including *N*-acetylcysteine, glutathione, and allopurinol, increase proteoglycan levels on Fn fragments as a result of a reduction of the catabolic cytokines TNF-α, IL-1, and IL-6 in vitro [73, 74]. Glucosamine and chondroitin sulfate mixtures also increase proteoglycan levels after Fn fragment administration to cultured cartilage [75]. Despite these modest, favorable effects on the damage caused by Fn fragments, none of these agents are currently used in clinical treatments of OA. Another agent, hyaluronan, is in clinical use for OA, mainly because patients" pain and joint function can improve on this drug [43]. One of the underlying mechanisms for its benefit could comprise preventing the catabolic effects of Fn fragments, because on Fn fragment injection into rabbit joints, hyaluronan upregulates proteoglycan levels and improves histological disease characteristics [72]. In addition, after treatment with Fn fragments, hyaluronan promotes proteoglycan levels, and decreases NO levels in cultured human articular cartilage from OA patients [76, 77]. Whether hyaluronan contributes to structural cartilage improvement in OA needs yet to be established in human patients.

Multiple sclerosis: fibronectin aggregates inhibit regeneration of myelin

MS is a chronic disease of the CNS. Although the pathogenesis is unknown, many factors are recognized to play a role in MS onset, including genetics and environmental factors, such as cigarette smoking, Epstein–Barr virus infection and vitamin D levels [78]. Pathological hallmarks are CNS inflammation, myelin degeneration (demyelination) and axonal loss, which clinically reflect as neurological disability. Progression of MS occurs in distinct patterns, ranging from rapid accumulation of disability in primary and secondary progressive MS to episodes of fulminant inflammation and recovery in relapsing-remitting MS. On demyelination, regeneration of myelin (remyelination) is attempted by oligodendrocyte progenitor cells (OPCs), which can differentiate into myelin-forming oligodendrocytes. However, remyelination ultimately fails in MS, despite the presence of OPCs [79, 80], leaving axons unprotected by myelin sheaths, and therefore vulnerable to further degeneration [81–83]. Temporary, dimeric Fn expression occurs on demyelination, but Fn aggregates in MS lesions [23]. Persistence of Fn, likely in the form of Fn aggregates, is involved in the pathology of chronic MS via (a) (chronic) stimulation of inflammation, and (b) direct inhibition of OPC maturation to oligodendrocytes (Fig. 2).

Inflammation in MS involves, among others, the entry of immune cells to the brain, and the pathological activation of CNS-resident microglia. Leukocyte invasion to the brain in relapsing-remitting MS requires migration across the blood–brain barrier. The blood–brain barrier contains endothelial cells and astrocytes [84], expressing Fn [20, 85]. In order to cross the blood–brain barrier, leukocytes express integrin α4β1, which mainly binds to vascular cell adhesion molecule 1 (VCAM1) [86], but also to the CS1-peptide, which

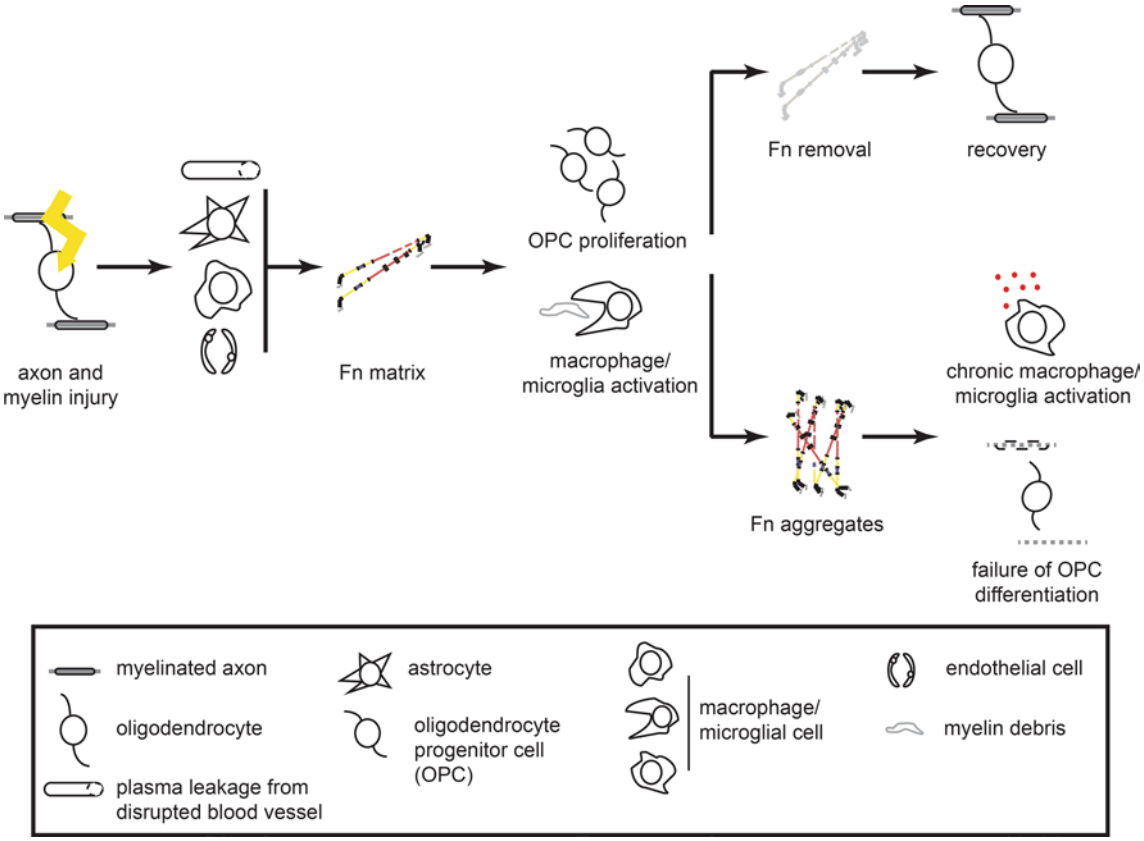

Fig. 2 On central nervous system injury in multiple sclerosis, improper fibronectin degradation is related to failure of remyelination and disease progression. Myelin injury initiates fibronectin (Fn) expression from plasma leakage, and secretion by astrocytes, macrophages/microglia, and endothelial cells (depicted from *top* to *bottom*, respectively). This Fn matrix promotes myelin regeneration via different mechanisms, including stimulation of oligodendrocyte progenitor cell (OPC) migration and proliferation. Complete removal of the Fn matrix corresponds to remyelination, whereas Fn aggregates mediate failure of remyelination by impairing OPC differentiation, and possibly via continuous macrophage/microglia activation

is a domain of Fn (a site within the IIICS-region) (Fig. 1) on endothelial cells, or can be expressed independently of Fn on astrocytes [87, 88]. The α4β1-Fn CS1 interaction is blocked by natalizumab, an approved drug for relapsing-remitting MS that was designed to prevent leukocytes from binding to VCAM1 [85]. Also, interferon β-1b, another drug for relapsing-remitting MS, inhibits the ability of T-lymphocytes to migrate via Fn on endothelial cells [89]. Therefore, Fn on endothelial cells contributes to leukocyte invasion in relapsing-remitting MS. Although cell migration and proliferation on Fn usually benefit tissue regeneration, these examples demonstrate that such physiological functions contribute to pathology under inappropriate circumstances. Of note, stimulation of cell proliferation by Fn also becomes pathological in cancer metastases, when cancer cells invade new tissue and proliferate there [90–92]. Further, Fn contributes to MS inflammation by instructing the CNS resident microglia and, possibly, invaded macrophages. Fn interacts with integrin α5β1 on microglia to enhance MMP-9 secretion [93]. Also, Fn may bind to TLR4 on microglia, as has been suggested for wound healing and OA before. Expression of TLR3 and -4 is upregulated in human MS lesions [94], with TLR4 primarily localized to microglia [94, 95]. In vitro, pFn activates microglial cells to secrete pro-inflammatory cytokines, including IL-1β [96], TNF-α, CXCL1, CCL3, and CCL5, and enhances phagocytosis by microglia [97]. Because these effects depend on the presence of MyD88, they likely result from TLR4 stimulation. Interestingly, in these studies, pFn was examined, suggesting that pFn contributes to TLR4-stimulation as well as EIIIA-containing Fn fragments [40]. Immune activated microglia likely facilitate remyelination in MS. For example, phagocytosis of myelin debris is necessary for complete remyelination [98] and moderate inflammatory activity enhances remyelination [99, 100]. Also, macrophage/microglia activation by pFn is neuroprotective in traumatic brain injury [101]. However, TLR4 stimulation by LPS induces indirect loss of OPCs and oligodendrocytes and neurodegeneration in vivo [95, 102]. Therefore, the overall effects of Fn–microglia interactions in MS remain to be established, as well as the effects of Fn aggregates.

Fn also directly affects OPCs. OPCs express a variety of integrin receptors during the (re)myelination process, including the Fn receptors αvβ1, αvβ3, αvβ5, and αvβ8 [103–105]. In vitro, coatings of pFn stimulate integrin αvβ1 to enhance OPC migration [106, 107]. In addition, αvβ3 integrin stimulation by physiological levels of platelet-derived growth factor A (PDGF-A) and Fn enhances OPC proliferation [108]. Further, integrin αvβ5 signaling is important for OPC differentiation [104]. These studies indicate that Fn promotes recruitment of OPCs in the demyelinated area via αvβ1, αvβ3, and αvβ5 integrins, benefiting remyelination. However, in vitro myelin formation of OPCs is impaired on pFn, mediated by β1-integrin signaling and mislocalized MMP-9 activity [109–111]. This impairment of OPC maturation initially plays a useful role in remyelination because it allows for precise timing of the remyelination process [82]. However, as soon as OPC recruitment has been completed, αv integrin expression decreases [112] and the Fn matrix should be degraded, allowing OPCs to proceed to form myelin. Indeed, downregulation of Fn precedes remyelination on toxin-induced demyelination [22, 23, 105]. In contrast, in chronic relapsing experimental autoimmune encephalomyelitis (cr-EAE), an animal model for relapsing-remitting MS, Fn aggregates in the lesion areas. Fn aggregates also persist in chronic demyelinated MS lesions, and inhibit CNS remyelination on toxin-induced demyelination in vivo [23]. The mechanism for remyelination impairment needs yet to be established, but could comprise the perturbation of oligodendrocyte process outgrowth, myelin-membrane-directed vesicular transport, and membrane microdomain formation, as has been shown for pFn [110, 111, 113, 114].

How Fn aggregates are formed in MS warrants further investigation. Organization of Fn into fibrils (fibrillogenesis) and, ultimately, assembly into a three-dimensional matrix is a well-balanced process during tissue development and regeneration (extensively reviewed in [115, 116]). Fn aggregation, as defined by deoxycholate (DOC)-insolubility, is likely the result of strong, noncovalent protein–protein interactions [117, 118] (our unpublished observations), and participation of other extracellular proteins in this matrix [118]. Fn aggregation may be appropriate during initial stages of tissue regeneration [115, 116, 119]. However, excessive Fn deposition and inappropriate remodeling contribute to scarring and fibrosis, and frustrate complete tissue regeneration [116, 120]. Under physiological circumstances, maintenance of the Fn matrix requires continuous Fn synthesis by cells [121], but in MS, Fn mRNA levels were undetectable in chronic demyelinated lesions, where Fn aggregates nonetheless persisted [23]. This suggests that inappropriate remodeling, rather than continuous Fn deposition, is crucial for Fn aggregation in MS lesions. Fn remodeling into aggregates is likely mediated by self-assembly, interaction with binding sites on other proteins as well as with cellular receptors (mainly integrin receptors), and local enzyme activity [115, 122, 123]. Because transglutaminase activity is proposed to be required for Fn aggregation [124, 125], and transglutaminase interactions with Fn are active in MS [126], this enzyme is one of the factors that may contribute to Fn aggregation in MS, but this requires further investigation.

Concluding remarks: timely removal of the fibronectin scaffold is necessary to complete the build

In the development of therapeutic strategies for the promotion of tissue regeneration, there is much focus on the initiating mechanisms of specific diseases, for example via the identification of gene expression patterns relevant to disease onset. The rationale behind this approach is that to unravel the disease pathogenesis will likely provide targets for stopping tissue degeneration by its cause. Alternatively, a more pragmatic strategy is to tackle persistent factors in the injury environment that hamper regeneration. Fn is such a factor. This review illustrates similarities among the responses to injury in different tissues in the creation of a Fn matrix. Fn initially facilitates regeneration of skin, cartilage, and myelin, mainly via stimulating the recruitment of inflammatory and regenerative cells. However, whereas Fn is totally removed before complete regeneration takes place, persistent structural variants contribute to failing regeneration in OA and MS. Mechanisms by which persistent Fn mediates regeneration failure differ between specific tissue types, but showed similarities, especially in their interaction with the immune system. Therefore, adequate clearance of the Fn matrix benefits regeneration, and incomplete degradation contributes to failure of tissue regeneration (Fig. 2).

The contribution of a residual Fn matrix in regeneration failure is not limited to the tissues that have been discussed in this review. For example, in myocardial infarction, the Fn matrix is necessary for myofibroblast recruitment and differentiation [127], but on incomplete clearance of the matrix, the EIIIA-containing Fn mediates adverse cardiac remodeling [128]. Also, accumulation of Fn fragments occurs during intervertebral disc degeneration [129], and further enhances disc degradation via stimulation of MMP expression [130, 131]. These examples further emphasize the benefit of complete degradation from the Fn matrix for tissue regeneration. This also underlines the importance of tightly regulated dynamic ECM expression in general [132], especially because collagen fragments and persistence of tenascin-C also contribute to OA pathology [133, 134]. Similarly, the high molecular weight variant of hyaluronan, present in MS lesions, inhibits remyelination [135].

In designing therapies to improve tissue regeneration, both the good and the bad sides of Fn can be taken into account. Taking advantage of the pro-regenerative properties of Fn, it can be attempted to speed up regeneration by exogenous administration of Fn. In designing such therapies, it is essential to consider the concept of "dynamic reciprocity", which refers to the importance of well-balanced receptor and ligand signaling in time. In this concept, Fn administration can only add to healing if its (integrin) receptors are still upregulated [38]. Despite a potential mismatch between Fn and its receptors in chronic wounds, a modest additional benefit has been demonstrated for Fn-based therapies here. For example, after wounding the skin from obese diabetic mice [136], and also on rat peritoneal injury [137], the PHSRN fragment from the 9th type III domain accelerates wound healing. This acceleration benefits from the physiological properties of Fn, including an increased fibroblast and keratinocyte adhesion and migration, wound contraction [136], and angiogenesis via integrin α5β1 on epidermal and endothelial cells [138, 139]. Similarly, pFn slightly accelerates wound healing in rats when topically applied onto skin wounds [140, 141], and when injected after incisional wounding in a dose-dependent manner [142]. In patients with persistent corneal epithelial defects, topical application of pFn shows modest beneficial effects on healing [143]. Wound dressings, including Fn-based therapies, benefit healing in carefully selected wounds, and only in a subset of wounded patients [144]. Therefore, the therapeutic potential of exogenous Fn application could further be enhanced by selecting specific patient groups, such as wounded patients with diabetes mellitus [141]. In addition, these therapies may be more effective when Fn domains are coupled to (a) other supportive proteins, such as hyaluronan [145], (b) growth factors, such as PDGF [140] and hepatocyte growth factor [146], or (c) glycoprotein hormones, such as erythropoietin [31, 147]. Finally, in another elegant approach, a fibrin/Fn matrix was designed, that could bind the growth factors PDGF, vascular endothelial growth factor (VEGF), and bone morphogenetic protein (BMP) to enhance healing of skin wounds of diabetic mice [148].

To overcome the detrimental properties of persistent Fn, multiple strategies are possible: (a) ensuring proper Fn clearance, (b) eliminating Fn structural variants once they emerge, and (c) by-pass of harmful Fn signals. We briefly discussed therapeutic strategies in OA and MS that, among their other actions, by-pass Fn signals. By-passing strategies included hyaluronic acid (OA), natalizumab, and interferon β-1b (MS), although these effects occur secondary to how the drugs were designed. These and the other approaches warrant further investigation. To accelerate our understanding, a multidisciplinary approach will be helpful, comparing good and bad sides of Fn and Fn therapies between tissues. This will expand our insight into how improper Fn clearance is mediated, and could be overcome. These insights will likely benefit therapeutic strategies that promote tissue regeneration.

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