Matricellular proteins as modulators of wound healing and the foreign body response

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Summary
Matricellular proteins form a group of extracellular matrix (ECM) proteins that do not subserve a primary structural role, but rather function as modulators of cell-matrix interactions (1). Members of the group, including thrombospondin (TSP)-1, TSP-2, SPARC, tenascin (TN)-C, and osteopontin (OPN), have been shown to participate in a number of processes related to tissue repair. Specifically, studies in knockout mice have indicated that a deficiency in one or more of these proteins can alter the course of wound healing. More recently, TSP1, TSP2, and SPARC have also been implicated in the foreign body response, an unusual reaction to injury that occurs after the implantation of biomaterials. This review will focus on the roles of these proteins in the response to injury in mice and will show how studies of this pathophysiological process can elucidate some of the intrinsic properties of these matricellular proteins.

Keywords
Matricellular proteins, wound healing, foreign body response, thrombospondin, cell-matrix interactions

Introduction

Extracellular matrix and matricellular proteins
Collagens, proteoglycans, and glycoproteins are the major constituents of the ECM, and collectively provide cells with biological information and a proteinaceous scaffold for adhesion and migration (2-4). This dual role of the ECM is achieved by the actions of different classes of molecules with overlapping properties. For example, by binding cytokines, proteases, and protease inhibitors, diverse proteins in the ECM act as a reservoir for a number of growth factors, and can influence cell migration and proliferation. However, molecules such as collagen and fibronectin serve primarily a structural role in the matrix through the maintenance of its structural integrity. Among ECM glycoproteins, a small group of proteins that includes TSP-1, TSP-2, SPARC, OPN, TN-C, TN-X and the CCN family members CCN-1 (cyr 61) and CCN-2 (connective tissue growth factor; CTGF) are thought to fulfill their biological function by binding to matrix proteins, as well as to cell-surface receptors and molecules such as proteases and cytokines (1). A detailed set of reviews regarding the properties of these proteins has been published (5-12).

Despite the well-documented expression of matricellular proteins during embryonic development, with the exception of the CCN proteins, mice that lack one or more of these proteins are viable and reproduce normally. The lack of a developmental phenotype prompted some investigators to study the process of wound healing and the foreign body response in adult null mice since it involves many aspects of embryonic development but lacks the complex repertoire of proteins required during development of an organism. Healing responses in these mice differed from those of their wild-type counterparts and, more
importantly, from each other. Collectively, the diverse and sometimes opposite effects on healing suggest a unique mode of action for each protein, which is determined not only by their intrinsic properties but also by the spatiotemporal context within which they function. Since, CCN1- and CCN2-null mice die in utero or shortly after birth (13, 14), these animals cannot be used to analyze wound healing or the foreign body response (FBR). However, both CCN1 and CCN2 have been shown to participate in wound repair (15-17).

**Wound healing**
Wound healing consists of a series of overlapping phases that includes inflammation, proliferation and migration, and remodeling (18). During the inflammatory response neutrophils, and then macrophages, provide a source for many important chemokines and cytokines that stimulate the migration and proliferation of repair cells such as endothelial cells and fibroblasts. These repair cells are responsible for the processes of angiogenesis and matrix deposition and remodeling that are required for wound healing progression and resolution. Cellular changes are accompanied by changes in the extracellular matrix, and within this dynamic environment cell-matrix interactions are critical. Specifically, cells that migrate into a wound have to remodel the matrix that surrounds them and secrete a new matrix that will constitute the neodermis. Neovascularization during healing is also a controlled process that initially involves an influx of blood vessels within the wound, followed by their subsequent regression (19). The mechanism by which regression occurs is unknown, but induction of endothelial cell apoptosis has been proposed as a possible explanation (20). Healing skin wounds in mice consist of a loose ECM, that is rich in blood vessels (Fig. 1A), but eventually recover enough tensile strength to regain their mechanical integrity. However, resolution of the wound healing response is generally not ideal because of scar formation and the failure to regenerate specialized structures, such as sweat glands and hair follicles. Thus, despite the formation of an adequate barrier, the functional and cosmetic recovery of the skin could be improved. Furthermore, because compromised wound healing is observed in certain pathological conditions such as diabetes, strategies that aim to improve healing are highly desirable.

**The foreign body response**
Like wound healing, the FBR also involves a number of distinct but overlapping biological processes, but these lead instead to the encapsulation of the foreign material (21, 22). The process is remarkably similar to the formation of immune granulomas that form as a result of infective granulomatous diseases, e.g. tuberculosis (20). In addition, the formation of foreign body giant cells (FBGC), i.e. fused multinucleated macrophages, is considered a hallmark of the FBR. These cells are responsible for the extensive surface damage that can be inflicted on implanted biomaterials (23). In normal wound healing the ingress of neutrophils and activated macrophages is transient and gives way to the proliferative and remodeling phases that complete wound repair (18). Thus, the wound healing response can be characterized as self-limiting since inflammation and remodeling cease following repair. On the contrary, in the foreign body response FBGC persist at the site of implantation, sometimes for years (23). Their presence indicates that the FBR involves a chronic inflammatory response that distinguishes it from wound healing. Overall, the presence of FBGC, coupled with the deposition of a collagenous capsule that is largely devoid of blood vessels, characterize the FBR (Fig. 1B).

The relative lack of blood vessels in capsules is an interesting feature of the FBR. Generally, healing is supported by blood vessel formation. For example, we have found the vascular density of wild-type wounds during peak remodeling to be $11 \pm$
4 vessels per high power field (hpf) (24). In the interstices of PVA sponges the number is much larger (40 ± 8 per hpf) (25). However, the FBR can proceed without a substantial influx of blood vessels (3 ± 1.5 per hpf in foreign body capsules) (26). To support this observation we have examined capsules at 2 and 4 wk post-implantation and found that their vascular densities were similar (unpublished observation). Thus, the influx of blood vessels followed by regression that occurs in wound healing and PVA sponges does not occur during the FBR. Due to the low vascular density of capsules, encapsulation can be a hindrance to implants that require communication with the host’s circulation for proper function (e.g. sensors and delivery devices) (27).

Capsules surrounding biomaterials can achieve great thickness (over 200 µm) and are characterized by the deposition of highly ordered collagen fibers (22). Unlike remodeled dermal wound tissue, capsules have a low cellular content. In fact, their density is increased by two-fold and diffusion rates of molecules through them have been shown to be reduced by more than half, in comparison to normal dermis (28). The makeup of the capsule suggests that the host’s aim is to isolate the implant from surrounding tissues (29). The combination of FBGC at the implant surface and the dense collagenous capsule that surrounds them provides a barrier that is maintained by signals initiated by the continuous presence of the implant.

**PVA sponges**

Implantation of polyvinyl alcohol (PVA) sponges can serve as an in vivo model with characteristics of both wound healing and the FBR. Specifically, in the early phase (1-3 weeks) the invasion of the sponge by cells and the formation of new tissue permits the estimation of neovascularization and fibroplasia that occurs during wound healing (30, 31). This is due to the enhanced neovascularization and loose organization of the collagenous matrix that is deposited within the interstices of the sponge. However, in wild-type mice the blood vessel density within sponges (25) is at least three-fold higher than in healing dermal wounds (24). In addition, the eventual encapsulation of the sponge and the presence of FBGC suggest that the model, in its later stages (after 3 wk), is indeed one of a foreign body response. Despite this increased complexity, the implantation of PVA sponges has proven to be a useful model for deciphering the role of some matricellular proteins in modulating both cell-matrix interactions and the properties of the extracellular matrix (25, 32).

### Matricellular proteins and wound healing

**Thrombospondins**

TSP1 and TSP2 are modular proteins that function at the interface between the cell surface and the ECM as a result of their ability to bind cell-surface receptors such as integrins, CD36, CD47, and proteoglycans, as well as bioeffector molecules such as proteases and growth factors (5). Both TSP1 and TSP2 have potent anti-angiogenic activities and can modulate the effective levels of matrix metalloproteinases (MMPs) (33). In addition, TSP1 has a role in the activation of transforming growth factor (TGF)-β1 (34), and studies in TSP2-null mice have established its participation in the regulation of collagen fibrillogenesis (35) and platelet formation (36). In TSP2-null mice, full-thickness excisional wounds healed at an accelerated rate and resolved with minimal scarring in comparison to wounds in their wild-type counterparts (24) (Table 1). Histological examination of the wound-healing process revealed abnormalities in the migration of the epithelium, the neovascularization of the wound bed, and in the organization of the ECM within the remodeling dermis. The latter, which consists of irregularly deposited collagen fibers, reflects the involvement of TSP2 in collagen fibrillogenesis and is consistent with the formation of irregularly shaped collagen fibrils in the skin and tendons of TSP2-null mice (35). This finding is surprising because TSP2 is not an

| Table 1: Wound healing-related phenotypes in knockout mice. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Healing rate    | Inflammation    | Character of the matrix | Angiogenesis | References |
| TSP2            | ↑ ↑             | NC¹             | Large irregular collagen fibrils | ↑ ↑         | 24,39      |
| TSP1            | ↓               | ↓               | NC              | NC            | 39-42      |
| SPARC           | ↑               | NC              | Small regular collagen fibrils | NC          | 47         |
| OPN             | NC              | ↓               | Small regular collagen fibrils | NC          | 49         |
| Tenascin-C      | NC              | NC              | Reduced fibronectin deposition | NC          | 50-52      |

¹NC, no change
integral component of collagen fibrils (37). Ultrastructural studies of developing flexor tendons in TSP2-null mice suggested that the deficiency involves the cellular processes that extend from collagen-secreting fibroblasts and organize the developing fibrils (38). Thus, TSP2 is required for fibril assembly following secretion of collagen into the extracellular space. Increased neovascularization results from an influx of vessels and reduced vascular regression, both of which may be associated functionally with increased levels of MMPs. Specifically, we have found that the levels of both MMP2 and MMP9 were significantly elevated in TSP2-null granulation tissue (unpublished observation). The peak in MMP levels in TSP2-null wounds coincided with the highest levels of TSP2 content in wild-type wounds and occurred during the remodeling phase (days 7-14) of wound healing (34, 39). Based on an immunohistochemical analysis of murine wounds, TSP2 appears to be secreted predominantly by wound fibroblasts and is transiently associated with the ECM, where it is well positioned to mediate vascular regression. Surprisingly, despite the reduced tensile strength of uninjured skin in TSP2-null mice, wounds recover their tensile strength normally (unpublished observation). Thus, local inhibition of TSP2 synthesis or function could accelerate healing and reduce scarring without compromising the integrity of healed wounds.

The distribution of TSP1 in murine skin wounds is distinct from that of TSP2 and is dominant during the early inflammatory phase (39). TSP1 is secreted by platelets and macrophages, and perhaps by the first endothelial cells and fibroblasts that enter the wound bed, but its presence in murine wounds cannot be detected after day 5 (39). However, in human skin wounds TSP1 was also detected by immunohistochemistry around blood vessels during the late stages of wound closure (40). Antisense-mediated inhibition of TSP1 during skin wound healing was shown to cause delayed healing (41). Surprisingly, transgenic mice that overexpress TSP1 also displayed delayed healing of full thickness excisional wounds (42). It is possible that the synthesis of TSP1 in an abnormal spatial and temporal context can have effects that are not associated with its normal function. We suggest that when TSP1 is overexpressed in late wounds it may function like TSP2, and thus delay healing.

A more convincing case for the role of TSP1 in skin wound healing was made in studies in TSP1-null mice, which displayed delayed wound healing associated with a reduced inflammatory response (39) (Table 1). Specifically, the levels of the CC chemokine ligand CCL2 (also known as MCP-1), and total and active TGF-β1, are reduced in these mice. In addition, the macrophage content of TSP1-null wounds was reduced, indicating that the presence of chemotactic factors may be compromised. In summary, the loss of TSP1, either by antisense technology or genetic deficiency, leads to delayed healing and is associated with a reduced inflammatory response. Thus, the roles of TSP1 and TSP2 in wound healing appear to be distinct.

It would be interesting to determine whether exogenous TSP1 or TSP2 can restore a normal healing phenotype in TSP2- and TSP1-null mice, respectively.

The availability of double TSP1/TSP2-null mice prompted us to examine the wound healing response in these animals (39). Like TSP1-null, double TSP1/TSP2-null mice display delayed healing due to a reduced initial inflammatory reaction. Apparently, the early loss of TSP1 and the reduced inflammatory response negated any beneficial effects that might have been expected from the loss of TSP2 during the remodeling phase. Thus, the increased angiogenesis and associated improvements that are seen in healing in TSP2-null mice were not observed in double-null animals.

**SPARC**

SPARC (secreted protein acidic and rich in cysteine; osteonectin; BM-40) is a matricellular protein that has been shown to inhibit cell-cycle progression and to induce de-adhesion in a variety of cell types (43). SPARC-null mice have a complex phenotype that includes early onset cataract formation (44, 45), osteopenia (46), and a reduced collagen content of skin (47). Two contradictory reports of the wound healing response in these mice have been published. Basu et al (48) reported delayed healing following the creation of 25 mm, but not 6 mm wounds, in SPARC-null mice. On the other hand, Bradshaw et al (47) observed accelerated healing of 5 mm wounds that was associated with the deposition of smaller than normal diameter collagen fibrils in the wound bed (Table 1). Because of this reduction in fibril diameter, it was postulated that the phenotype could be due to enhanced contraction of wounds. In view of the exceptionally large size of the wounds created by Basu et al (48), the interpretation of their results is difficult. Interestingly, both studies reported that the ability of SPARC-null primary dermal fibroblasts to contract artificial collagen gels was not compromised, suggesting that any abnormality in wound healing resulted from changes in the matrix.

**Other matricellular proteins**

Adult mice with targeted disruptions of other matricellular protein genes do not display alterations in the rate of healing, although minor histological abnormalities have been noted (Table 1). In day 14 wounds of OPN-null mice, collagen fibrils in the deep layers of the wound are reduced in diameter (49). In addition, OPN-null wounds are subject to a reduction in wound debridement despite normal macrophage levels, suggesting that the latter cells may not be functioning optimally. Tenascin-C-null mice accumulate reduced levels of fibronectin in day 13 excisional wounds (50). The deposition of tenascin-C at the margins of wounds (51) suggests that it might play a role in wound contraction and in vitro evidence indicates that its activity is mediated through an inhibitory effect on the activation of the GTPase RhoA in fibroblasts (52). However, these findings...
have not been validated in vivo. Tenascin-X-null mice manifest a phenotype that resembles a form of Ehlers-Danlos syndrome (53), and is similar to that of TSP2-null mice in some respects. Specifically, these mice exhibit increased skin and tendon laxity as a result of reduced collagen deposition, but the presence of additional abnormalities associated with the TSP2-null state, such as increased angiogenesis and a bleeding diathesis, has not been reported.

Collectively, studies of wound healing in mice that lack a matricellular protein indicate the ability of these proteins to influence all phases of healing, and a wide range of processes that include inflammation, cell migration, angiogenesis, and matrix deposition. As suggested by numerous in vitro studies, these alterations are likely to result from a perturbation of cell-matrix interactions and alterations in the levels of proteases and growth factors in the wound bed.

### Matricellular proteins and the FBR

#### Thrombospondins

In view of the significant overlap that exists in the pathophysiology of wound healing and the FBR, we hypothesized that the latter might also be altered in mice that lack one or more matricellular proteins. The deposition of TSP2 within implanted PVA sponges (25) and in the foreign body capsule (26) has been shown to be dynamic and to persist for up to at least 4 wk. Furthermore, studies in mice that lack TSP2 yielded capsules surrounding silicone disks that displayed a 6-fold increase in vascular density, and a 1.5-fold increase in thickness, and consisted of loosely packed irregular collagen fibers (26) (Table 2). The increased blood vessel density of capsules in TSP2-null mice suggested that the reduced blood vessel content of capsules in wild-type mice results, to a large extent, from the lack of the potent anti-angiogenic activity of TSP2. A similar observation was made in the PVA sponge granuloma model. In addition, MMP2 levels were elevated in sponges from TSP2-null mice. The observed increase in MMP2 is consistent with our previous findings in TSP2-null dermal fibroblasts (54). On the contrary, the levels of active TGF-β1 in sponges from TSP2-null mice were normal. A model involving the activation of TGF-β1, by the relative levels of TSP1 and TSP2, has been suggested (55). Based on this model, binding of TSP2 to TGF-β1 can prevent its activation by TSP1. Our studies in PVA sponges, in which the expression of TSP1 and TSP2 overlap, indicate that TSP2 is not critical for the regulation of the activity of TGF-β1.

The above findings raise the possibility that modulation of TSP2 levels could represent a means by which capsule vascularization can be increased. Indeed, anti-sense TSP2 gene experiments confirmed the ability of TSP2 to modulate neovascularization and matrix assembly in the FBR (56). Interestingly, a similar approach was successful in improving dermal healing in wild-type mice (57). The loose structure of the ECM that is associated with a deficiency in TSP2 may also help in reducing the contraction-mediated misshaping of malleable implants, e.g. breast implants. Taken together, studies of the FBR in TSP2-null, and in wild-type mice with locally inhibited synthesis of TSP2, demonstrate the importance of this matricellular protein in the formation of new tissue as a response to injury in an adult animal.

Preliminary analyses of PVA sponges implanted in TSP1-null mice suggest that their response was remarkably similar to that of TSP2-null mice (unpublished observations). Thus, unlike wound healing, the response was characterized by abnormal matrix deposition and increased angiogenesis (Table 2). A possible explanation for the discrepancy could be the prolonged synthesis of TSP1 in sponge granulomas, which persists for at least 3 wk. Secreted by macrophages that are resident in chronic granulomas, the continued presence of TSP1 within the interstices of the sponge could continue to exert an anti-angiogenic activity. This observation highlights a major difference between the wound healing and sponge implantation models. Since the latter is dominated by continuous inflammatory response, the prolonged expression of inflammation-associated proteins distinguishes this model from wound healing. Furthermore, FBGC can be observed within the sponge as early as day 7. Thus, the interpretation of results regarding the function of proteins, especially those involving angiogenesis, should take such differences into consideration.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Capsule thickness</th>
<th>Inflammation</th>
<th>Character of the matrix</th>
<th>Angiogenesis</th>
<th>References</th>
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<td>TSP2</td>
<td>↑↑</td>
<td>NC¹</td>
<td>Irregular collagen fibers</td>
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<td>SPARC</td>
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<td>NC</td>
<td>Small regular collagen fibers</td>
<td>↓</td>
<td>58</td>
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¹NC, no change
SPARC
Analysis of the FBR in SPARC-null mice indicated that capsule formation was compromised and, consistent with the findings in dermal wounds, was associated with the formation of smaller diameter collagen fibrils (58) (Table 2). In addition, even taking into consideration their reduced thickness, the capsules in SPARC-null mice were less vascularized. The authors suggest that a minimal threshold of collagen deposition, higher than that observed in these mice, has to be achieved to promote normal angiogenesis.

Other matricellular proteins
A deficiency in osteopontin results in changes in a specialized form of the foreign body response, such as that observed in ectopic calcification of glutaraldehyde-fixed aortic valve leaflets (59). Specifically, OPN-null mice showed accelerated and greater calcification of the leaflets in a subcutaneous implantation model. In the same study, it was demonstrated that OPN inhibits mineral deposition and actively promotes its dissolution. A role for OPN in the inhibition of vascular calcification has also been suggested (60). Since OPN can influence the activity of macrophages (61), and is expressed both during wound healing (62) and in the subcutaneous implantation of biomaterials (59), it is likely that the protein participates in the FBR (Table 2). An analysis of the response to biomaterials should be informative.

Conclusion
The analysis of in vivo models of wound healing and the FBR has proven useful in studying the function of matricellular proteins in mice that are null for one or more of these proteins. This approach has also presented investigators with an opportunity to identify new functions for these proteins, and to verify the findings of in vitro experiments. Thus, despite the early disappointment associated with the lack of clear developmental phenotypes in these mice, the studies outlined in this review have established a role for matricellular proteins in the tissue response to injury. In addition, much has been gained in our understanding of the complex interplay between these proteins and matrix-degrading enzymes, and their role in ECM assembly. At the same time, a number of important clues regarding the modulation of the FBR and wound healing have been obtained. For example, we now have potential targets, such as TSP2 and SPARC, for the improvement of the healing response in skin (accelerated healing and reduced scarring) and the FBR (increased angiogenesis, reduced capsule density and thickness). In the case of the FBR, the elucidation of the role of matricellular proteins has generated a new paradigm that shifts the focus towards the host response in efforts to improve biocompatibility (63). We expect that the continued investigation of the role of the matricellular proteins, and their associated molecules, in the FBR will lead to the identification of precise molecular signals that can be applied to the intelligent design of biocompatible biomaterials and scaffolds that are intended for tissue engineering applications.

Acknowledgments
Studies from the authors’ laboratory were supported by grant AR45418 from the National Institute of Health and by the University of Washington Engineered Biomaterials Engineering Research Center (NSF grant EEC9529161).