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Opportunities and Risks of Biofunctionalization of Implants with Extracellular Matrix Protein 1 to Support Skin and Bone Healing

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Abstract

Extracellular matrix protein-1 (ECM-1) plays a crucial role in the activation of latent transforming growth factor-beta (TGF-β). Active TGF-β governs key phases of tissue repair including inflammation, fibroblast activation, angiogenesis, and matrix remodeling. Dysregulation of TGF-β signaling is a hallmark of chronic wounds, excessive scar formation, and fracture non-union. As a protein of the extracellular matrix, ECM1 offers ideal conditions for the biofunctionalization of implants. Its mode of action has been studied mainly in fibrosis models, where TGF-β acts as driver of the disease. Controlled knock-out or over-expression of ECM1 either promoted or improved fibrosis development. In this review, we discuss how the findings can be applied to the biofunctionalization of implants to support the healing of skin and bone.

Keywords: wound healing; chronic wounds; non-unions; TGF-β; ECM1; angiogenesis; extracellular matrix

1. The Clinical Burden of Fractures, Chronic Wounds, and Dysregulated Healing

The burden of impaired bone and wound healing is a major clinical and socioeconomic challenge, particularly in elderly and multimorbid populations. Fractures and immobility-related pressure ulcers create a massive treatment demand worldwide [1–4]. Despite increasing knowledge about the cellular and molecular mechanisms of the underlying healing phases, 2–10% of all fractures result in delayed healing or the formation of non-unions [5,6]. Proximal femoral fractures, complicated by chronic ulceration, are common scenarios linking bone healing and soft tissue repair [7–9]. Chronic wounds affect up to 2% of the elderly population and are associated with high mortality, loss of independence, and spiraling healthcare costs [10–12]. Failed healing results in significant pain and functional impairment of the affected extremity, reducing the quality of life for the affected patient. Furthermore, the lengthy rehabilitation process, as well as the loss of labor and productivity, represent a significant economic burden on our healthcare system [13,14]. A detailed understanding of the molecular regulation of wound healing is thus paramount.

At the heart of wound healing lies the extracellular matrix (ECM), a dynamic environment regulating cell migration, angiogenesis, fibroblast activation, and scar formation [15–17]. Growth factor–ECM interactions, particularly via transforming growth factor-β (TGF-β), determine the balance between regenerative versus fibrotic outcomes [18–21]. While collagen, elastin, and fibronectin are heavily studied components of the ECM, more recently extracellular matrix protein 1 (ECM1) has emerged as a pivotal but underappreciated regulator of tissue repair and regeneration.

This review will delineate ECM1's role in wound healing through modulation of the TGF-β signaling pathway and highlight the connection of immune, fibrotic, and angiogenic control in wound and fracture healing.

2. TGF-β Signaling in Wound and Fracture Healing: Friend and Foe

Wound and fracture healing are coordinated in sequential but overlapping phases: hemostasis, inflammation, proliferation (granulation tissue, angiogenesis, fibroblast activity), and remodeling/maturation [22–24]. TGF-β is central in orchestrating this process:

During the **inflammation phase** large amounts of active TGF-β are released by coagulating platelets in order to recruit immune cells and modulate macrophages phenotypes [25,26]. In the following course of healing TGF-β gets activated from the extracellular reservoir of latent TGF-β either by proteolytic cleavage from latent TGF-β binding protein (LTBP) or by mechanical forces or acidification of pH. In the **proliferation phase** TGF-β stimulates fibroblast differentiation into myofibroblasts [27], promotes angiogenesis [28,29], and regulates ECM deposition [18]. In the **remodeling phase**, balanced TGF-β-activity ensures proper ECM turnover via orchestrating collagen deposition and metalloproteinase activity [30,31].

While TGF-β signaling is associated with impaired bone and wound healing [32–35] its prolonged or enhanced signaling is associated with fibrotic conditions, keloid, hypertrophic scar formation and chronic wounds [19,31,36]. Thus, regulation of TGF-β is essential to steer repair toward regeneration rather than fibrosis.

3. Extracellular Matrix Protein 1 (ECM1)

3.1. EMC1: a Multifunctional ECM Protein in Skin and Wound Repair

Extracellular matrix protein-1 (ECM1) is a glycoprotein secreted into the ECM, expressed by keratinocytes, fibroblasts, and endothelial cells [37]. It regulates skin barrier integrity, angiogenesis, keratinocyte differentiation, and matrix organization [38]. Its clinical relevance is underscored by Lipoid Proteinosis, a genetic disease caused by ECM1 mutations, characterized by scarring and impaired skin integrity [39].

In wound healing, ECM1 has been shown to (i) Modulate angiogenesis by interacting with VEGF and perivascular ECM components [17,40]; (ii) Bind to perlecan and influence basement membrane assembly and keratinocyte migration [28,41]; (iii) regulate matrix turnover by inhibiting MMP-9 activity, thereby balancing ECM degradation and deposition [17,42].

ECM1 functions as an integral mediator balancing angiogenic activity and fibrotic suppression, both of which are tightly regulated by TGF-β–dependent molecular mechanisms ([15,17,41–44])

Moreover, ECM1 deficiency correlates with abnormal myofibroblast persistence, excessive collagen I deposition, and impaired re-epithelialization – all classically associated with TGF-β dysregulations [31,45].

3.2. Interplay of ECM1 with TGF-β Pathway

TGF-β, with its three iso-forms (TGF-β1, -β2 and -β3), is by far the most abundant cytokine within the TGF-β superfamily. TGF-β is expressed as a latent protein, the so-called latency-associated peptide (LAP). The LAP is covalently bound to LTBP through disulfide bonds in the endoplasmic reticulum. The resulting large latent complex (LLC) is then incorporated into the extracellular matrix, waiting to be activated either enzymatically by proteolytic cleavage, chemically by a drop in pH, or mechanically by interaction with integrins [46]. The released active TGF-β dimers transduce their signals through two types of serine/threonine kinase receptors, termed type I and type II [47]. The type II receptors are constitutively active kinases, which phosphorylate type I receptors upon ligand binding. Seven type I receptors termed activin receptor-like kinase (Alk)-1 through -7 were identified in mammals, of which TGF-β1-3 preferably bind Alk-4 and Alk-5. Upon activation by the type II receptor, Alks activate (phosphorylate) Smad transcription factors in the cytoplasm. TGF-β1-3

activated signaling is mainly mediated via the receptor-regulated Smads (R-Smads) -2 and -3, which upon phosphorylation complex with the common-partner Smad (Co-Smad) 4 to enter the nucleus and regulate expression of target genes, including genes participating in feedback mechanisms of the signaling pathway itself [32,47] – for overview see Figure 1.
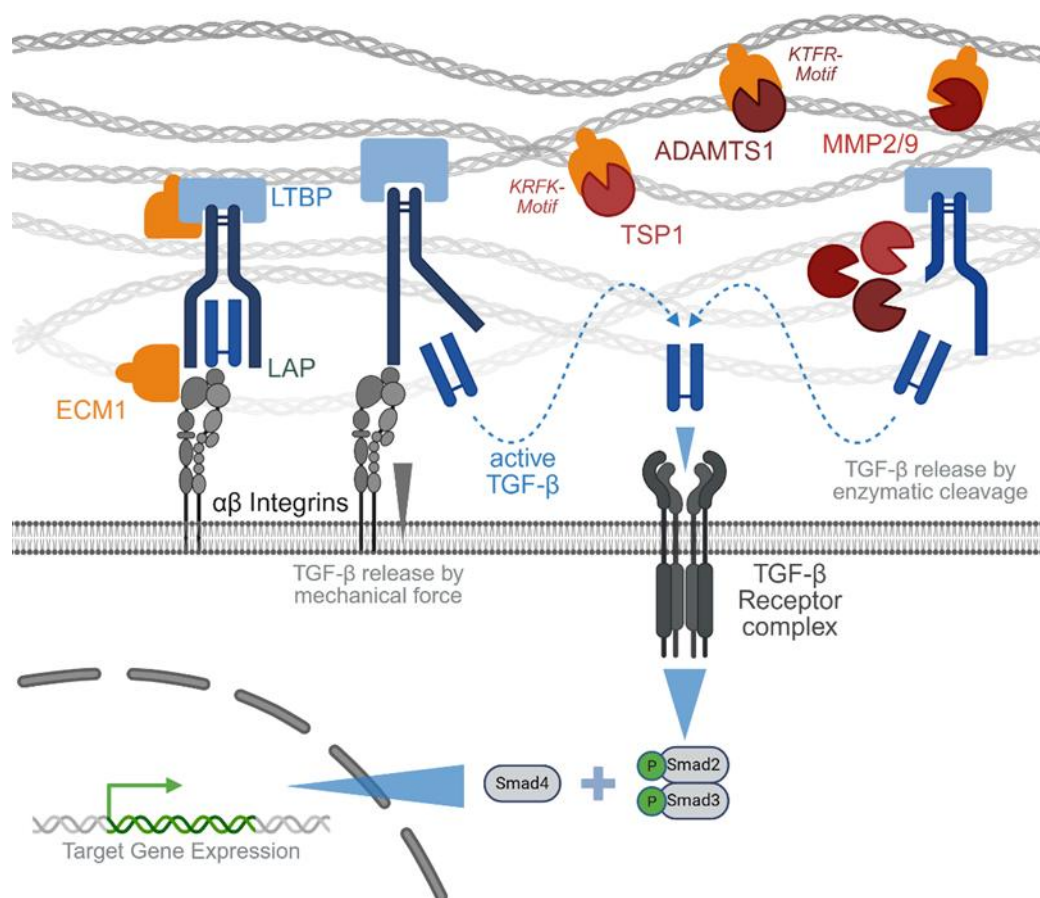

Figure 1. ECM1 as extracellular regulator of TGF-β Signaling – proposed modes of action. The extracellular matrix protein 1 (ECM1) is incorporated in the extracellular matrix. There are different modes of action described of how ECM1 may regulate transforming growth factor beta (TGF-β) activation. ECM1 may prevent the release of active TGF-β from the latency-associated peptide (LAP) (i) by strengthening its binding to the latent TGF-β binding protein (LTBP) or (ii) by inhibiting the mechanical opening of LAP by αβ integrins. (iii) Further, ECM1 was reported to bind proteases, e.g. a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1 – at its KTRF-motif), matrix metalloproteinase 2 and 9 (MMP2 and MMP9), and thrombospondin 1 (TSP1 – at its KRFK-motif), involved in the proteolytic release of active TGF-β from the LAP. Active TGF-β dimers, can then bind the TGF-β receptor complex to phosphorylate the intracellular transcription factors small mothers against decapentaplegic 2 and 3 (Smad2 and Smad3), which upon binding to Smad4 enter the nucleus to regulate target gene expression. This figure was created in BioRender. To access the original source of this figure, visit: https://app.biorender.com/illustrations/671f5c9f83adf666c3253635.

Mechanistically, ECM-1 modulates TGF-β through regulation of the ECM scaffold and growth factor bioavailability. By binding matrix proteoglycans and interacting with collagen/elastin networks [41–43], ECM-1 influences how latent TGF-β binding proteins (LTBPs) store and present TGF-β in tissues [44]. There are different modes of actions described [48]. Integrins, such as αvβ3, αvβ5, and αvβ6, can mechanically activate TGF-β by binding connecting the LLC to the contractile cytoskeleton. This requires binding of the Integrins to the RGD (arginine-glycine-aspartic acid) motif in the LAP. It has been reported that ECM1 protects TGF-β from activation by competitively binding to this RGD sequence, thus preventing the interaction of LAP with the integrins [48]. ECM1 was further reported to interfere with the proteolytic activation of TGF-β by blunting the activity of matrix

metalloproteinases 2 and 9 (MMP2 and MMP9), ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs 1), and TSP1 (thrombospondin 1) by interacting with their intrinsic KTRF (lysine-tryptophan-arginine-phenylalanine) and KRFK (lysine-phenylalanine-arginine-lysine) motifs, respectively [40].

4. Proposed ECM1 Effects During Wound and Fracture Healing

Targeting the ECM1 as extracellular regulator of TGF-β signaling offers potential therapeutic strategies, as ECM1 supplementation via biomaterials or gene therapy could stabilize wound ECM, facilitate controlled angiogenesis, and restore growth factor presentation. However, the proposed effects strongly depend on the healing phase and the predominant TGF-β isoform involved [49]. While TGF-β1 and TGF-β2 produce similar effects, the effect of TGF-β3 is partly opposite.

Tissue injury typically involves rupture of blood vessels. The resulting exposure of platelets (thrombocytes) to subendothelial collagen leads to platelet aggregation, degranulation and activation of the coagulation cascade. The formed fibrin clot both stops the bleeding and serves as a scaffold for the migration of inflammatory cells into the injured tissue. Upon activation platelets release the content of their granula. Platelet alpha granules are a particularly rich source of active TGF-β isoforms, especially of active TGF-β1, which is up to 100-fold more abundant than in other cell types (TGF-β1 : TGF-β2 : TGF-β3 ratio is 4000 : 1 : 10) [50]. This results in a rapid and strong local increase in active TGF-β isoforms at the site of tissue damage early during hemostasis. The release TGF-β1 and TGF-β2 act as potent chemoattractants and inflammatory mediators for various types of immune cells, such as neutrophils, mast cells and monocytes (Figure 2). In the invading neutrophils the ratio of TGFβ isoforms is biased towards TGF-β3 (TGF-β1 : TGF-β2 : TGF-β3 ratio is 12 : 1 : 34) [51], which is partly antagonizing the effects of TGF-β1 and TGF-β2 advancing the healing process towards the proliferation phase (Figure 3). As during hemostasis and the initial inflammation phase active TGF-β is released by platelets and neutrophils only very mild or even no antagonizing effects of ECM1 are expected. This is in contrast to the following healing phases, which require activation of TGF-β from the ECM.
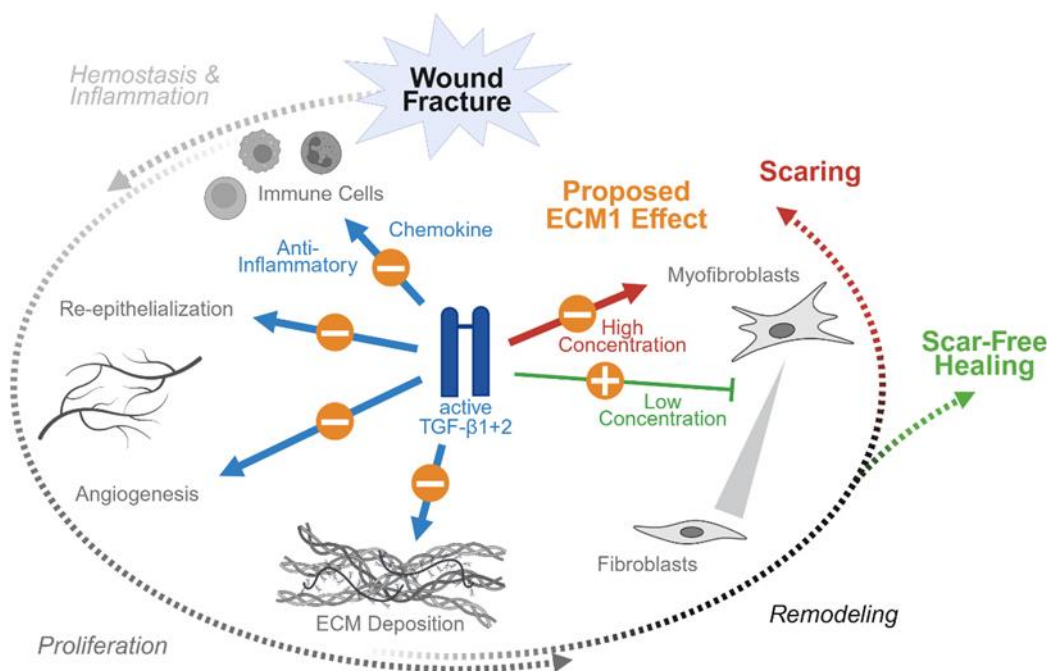

Figure 2. Proposed ECM1 effects during the different phases of wound and fracture healing dependent on its interaction with TGF-β1 and TGF-β2. The three different transforming growth factor beta (TGF-β) isoforms exert specific functions during wound and fracture healing in humans. In the initial phase of hemostasis and

inflammation, TGF-β1 and TGF-β2 act as chemokine to recruit immune cells to the wound/fracture. With their anti-inflammatory function they advance the healing process towards the proliferation phase. In the proliferation phase they promote re-epithelialization and angiogenesis, as well as extracellular matrix (ECM) deposition. In the following remodeling phase TGF-β regulates the transition from fibroblasts to myofibroblasts, however this effect is strongly dose dependent. While suppression of TGF-β1 and TGF-β2 supports scar free healing, its induction may favor scar formation by excessive activation of myofibroblast. The orange circles provide the proposed positive (+) or negative (-) effect of extracellular matrix protein 1 (ECM1), considering that it suppresses activation of TGF-β1 and TGF-β2 This figure was created in BioRender. To access the original source of this figure, visit: https://app.biorender.com/illustrations/671f5c9f83adf666c3253635.

In the proliferation phase three major events are mediated by TGF-β, namely re-epithelialization, angiogenesis, and formation of ECM. All three TGF-β isoforms have been reported to promote re-epithelialization by inducing proliferation and migration of epithelial cells at the wound margins [51,52]. However, with one exception for *in vitro* experiments, where keratinocyte migration was promoted only by TGF-β1 but not by TGF-β3 [53]. During the following angiogenesis, the endothelial cells form capillary sprouts that invade the wounded tissue to form a de novo microvascular network. The role for TGF-β as a modulator of angiogenesis is strongly context dependent and includes the recruitment of vascular endothelial growth factor (VEGF)-producing hematopoietic effector cells to the site of tissue damage, local induction of VEGF expression [28,54] and induction of endothelial to mesenchymal transition [55]. However, endothelial to mesenchymal transition has also been widely associated with pathological fibrosis of various organs, including the skin [56]. Finally, all three TGF-β isoforms participate in fibroblast recruitment to the site of injury and their activation to produce the provisional ECM, however, this process is strongly isotype dependent. TGF-β1 is reported to induce collagen production, specifically collagen type I and III. Hence, excessive TGF-β1-mediated signaling has been associated with scaring and the development of keloids [57]. The less abundant TGF-β2 shows similar effects than TGF-β1, unlike TGF-β3 which appears to be anti-fibrotic [58].

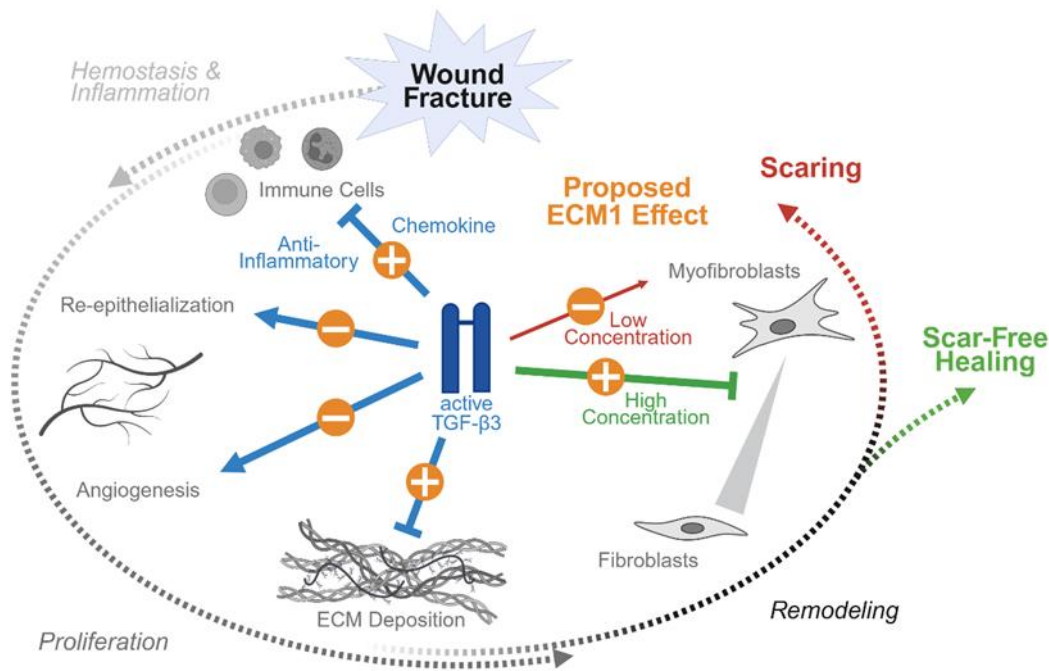**Figure 3.** Proposed ECM1 effects during the different phases of wound and fracture healing dependent on its interaction with TGF-β3. Transforming growth factor beta (TGF-β) effects on wound and fracture healing are dependent on the specific TGF-β isoform. In the initial phase of hemostasis and inflammation, the effect of TGF-β 3 is contrary to that of TGF-β1 and TGF-β2. It suppresses the recruitment of immune cells to the

wound/fracture. In the proliferation phase TGF-β3 supports re-epithelialization and angiogenesis, but unlike TGF-β1 and TGF-β2 inhibits extracellular matrix (ECM) deposition. In the following remodeling phase TGF-β3 regulates the transition from fibroblasts to myofibroblasts in a dose-dependent manner, where high levels of TGF-β3 are required for scar free healing. The orange circles provide the proposed positive (+) or negative (-) effect of extracellular matrix protein 1 (ECM1), considering that it suppresses activation of TGF-β. This figure was created in BioRender. To access the original source of this figure, visit: https://app.biorender.com/illustrations/671f5c9f83adf666c3253635.

This isotype-specific effect is enhanced in the following remodeling phase where TGF-β regulates the transition from fibroblasts to myofibroblasts, a population of fibroblasts with contractile phenotype. However, this effect is strongly dose dependent. Myofibroblasts are characterized by the expression of alpha smooth muscle actin (αSMA), which is controlled by TGF-β1, both through SMAD-dependent and independent signaling. Thus, suppression of TGF-β1 at this phase of healing supports scar free healing while its induction may favor scar formation by excessive activation of myofibroblast [27]. Similar to TGFβ1, TGFβ2 promotes the transition of fibroblasts to myofibroblasts both in vitro and in vivo (Figure 2). The role of TGF-β3, however, is more complex (Figure 3). It appears to promote myofibroblasts transition in vitro but inhibits the same process in vivo [58,59].

Especially in the proliferation and remodeling phase, where TGF-β is activated from the ECM reservoir, a controlled regulation of this process offers multiple options for intervention. ECM1 might establish as an ideal candidate for that, due to its extracellular field of action. Thus it is feasible that timely controlled ECM1 knock-down or neutralization might favor re-vascularization, angiogenesis and ECM deposition, resulting in an accelerated wound closure or fracture healing, as well as a stronger anchoring of implants in the bone. While later in the remodeling phase a controlled ECM1 induction might prevent scar or keloid formation. However, these timely effects require specific biofunctionalization strategies, discussed in the following paragraph.

5. Biofunctionalization Strategies

5.1. Protein-, RNA- and DNA-Based Strategies

Biofunctionlalization of implants or hydrogels to induce or neutralize ECM1 effects might be achieved by several strategies, including application of the recombinant human ECM1 protein directly or tetrapeptides, KTRF and KRFK, regulating its interaction with the proteases ADAMTS1 and TSP1 [40]. Further, Implants might be coated with RNA or DNA products to either induce or suppress ECM1 expression in the surrounding tissues (Figure 4). While RNA-based methods primarily influence gene expression at the post-transcriptional level [60], DNA-based strategies typically affect transcription at the genetic or epigenetic level [61].
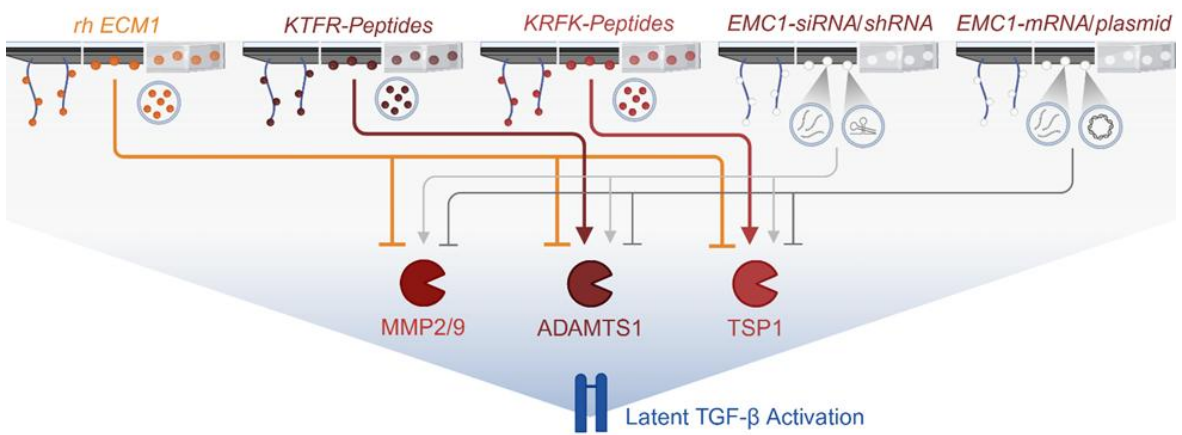

Figure 4. Examples for the biofunctionalization of implants through ECM1. As a protein normally situated in the extracellular matrix, extracellular matrix protein 1 (ECM1) is an ideal candidate for the biofunctionalization

of implant as its mode of action is within the extracellular space. Considering its proposed modes of action, there are different strategies are conceivable. Directly, by utilizing the recombinant human ECM1 (rh-ECM1) or the tetra-peptides KTFR (lysine-threonine-phenylalanine-arginine) and KFRK (lysine-phenylalanine-arginine-lysine). Or indirectly, using RNA or DNA based techniques, e.g. small interfering RNAs (siRNAs), small hairpin RNAs (shRNAs), aptamers, messenger RNA or plasmids, for either knock-down or overexpression of ECM1. These might be applied onto the implants either directly or with the help of polymers or vesicles or integrated into hydrogels. Based on the chosen strategy the applied protein/peptide, RNA or DNA may cause activation or inhibition of the proteases a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS1), matrix metalloproteinase 2 and 9 (MMP2 and MMP9), and/or thrombospondin 1 (TSP1), involved in the proteolytic release of active TGF-β from the LAP. This figure was created in BioRender. To access the original source of this figure, visit: https://app.biorender.com/illustrations/671f5c9f83adf666c3253635.

Induction of ECM1 expression might be achieved by applying synthetic messenger RNA (mRNA) or DNA-based strategies. Based on the fact that the biological stability for DNA is longer than for RNA, the administration of synthetic mRNA is suitable for a short and temporary induction of gene expression [60]. Importantly, DNA-based gene-delivery may alter the genome itself, depending on the method used – While the use of episomes and plasmids (circular DNA) allows for transient expression of the transferred gene, the use of transposons ("jumping genes"), CRISPR-Cas9 gene editing, and CRISPR activation (CRISPRa) leads to integration of the gene into the genome, allowing for stable expression of the transferred gene [61]. When using viral gene delivery, it strongly depends on the viral system used, if the delivered gene integrates (i.a. retroviral or lentiviral based gene-delivery with stable gene expression) or not (i.a. adeno-associated viral gene-delivery with transient gene expression) into the hosts genome [62].

Suppression or knock-down of ECM1 expression might be achieved by various RNA-based techniques, such as RNA interference (double-stranded RNAs) using small interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs), single-stranded microRNAs (miRNAs), long non-coding RNAs (lncRNAs), or aptamers (short single-stranded RNA or DNA molecules) [60], or DNA-based strategies. However, the later usually applied methods such as CRISPR-Cas9 gene editing or CRISPR interference (CRISPRi).

5.2. Alterations in TGF-β Levels Control Phyiological Wound and Fracture Healing ao

As described before physiological wound and fracture healing requires a burst release of active TGF-β from platelets leading to a steep increase in active TGF-β levels at the site of tissue damage during the initial hemostasis [50]. During the inflammatory phase infiltrating neutrophils then provide more active TGF-β, but with altered isotype composition, inducing negative feedback mechanisms which result in a continuous decline of active TGF-β levels down to basal levels [51] (Figure 5A).

When the initial burst increase in active TGF-β levels at the site of tissue damage is disrupted, the subsequent healing cascade may not be adequately initiated, leading to delayed or impaired wound and fracture healing [63]. This might have different reasons, such as smoking or chronic inflammation. Interesting, although smokers were reported to have increased number of platelets as compared to non-smokers and nicotine possesses the ability to active platelets [64], smoking has been associated with a suppressed increase in active TGF-β after trauma (Figure 5B1) [64]. The situation is different in patients with chronic inflammation, where high levels of active TGF-β are continuously secreted by immune cells. This is mainly observed in obese patients, patients with diabetes mellitus [65–71], or patients with fibrotic diseases of the liver, kidney, heart of other tissues [72–74]. The resulting elevated basal active TGF-β levels limit the local increase at the site of tissue damage (Figure 5B2).

Likewise, when the resolution of inflammation fails, the resulting prolonged activation of TGF-β may lead to increased scar or keloid formation [19], or suppress bone formation by impairing mechanotransduction in osteogenic cells (Figure 5B3) [75].
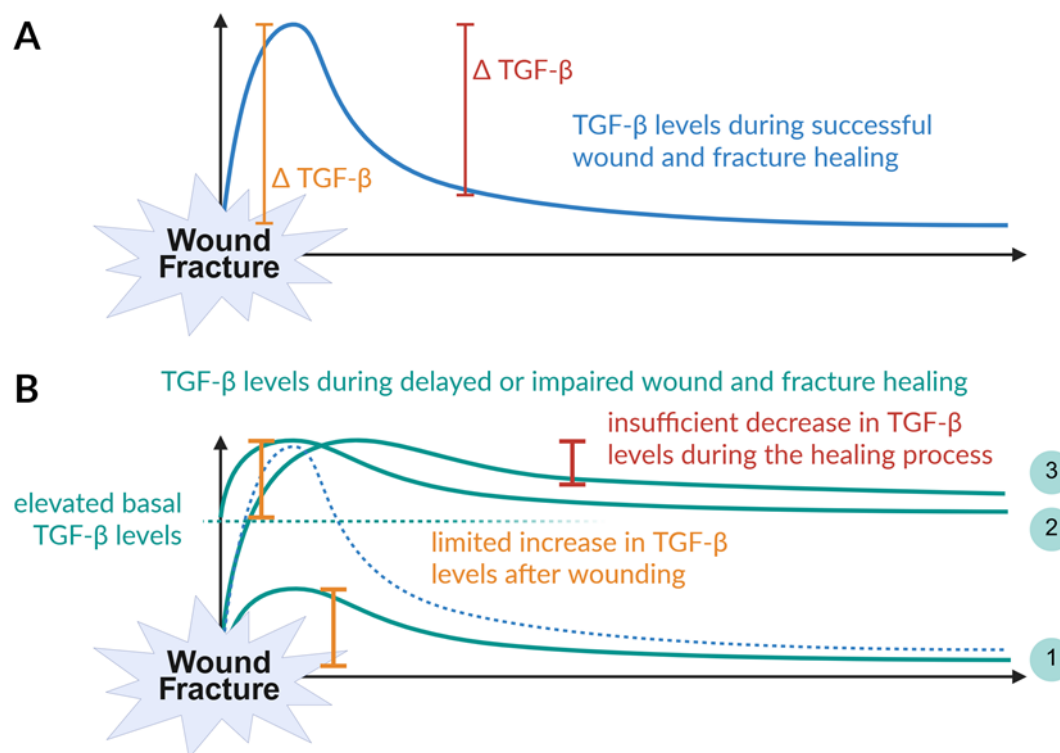
Figure 5. Alterations in active TGF-β levels in wound and fracture healing. (A) During physiological wound and fracture healing there is usually a burst release of active TGF-β from platelets leading to a steep increase (Δ) in active TGF-β levels at the site of tissue damage. Infiltrating neutrophils then provide more active TGF-β, but with altered isotype composition, inducing negative feedback mechanisms which result in a continuous decline of active TGF-β levels down to basal level. (B) during delayed or impaired wound and fracture healing this controlled release of active TGF-β may be disturbed. The healing process may not be initiated when the initial raise in active TGF-β levels is too low, either through inhibition, such as observed in smokers (1) or through already increased basal levels, such as observed in patients with chronic inflammation (2). Further a prolonged activation of TGF-β may lead to increased scar or keloid formation (3). This figure was created in BioRender. To access the original source of this figure, visit: https://app.biorender.com/illustrations/671f5c9f83adf666c3253635.

5.2.1. Proposed Use of Recombinant Human ECM1

Recombinant human ECM1 at the implant surface should prevent excessive TGF-β activation. The early hemostasis and inflammatory phase might not be affected by such a coating, as there active TGF-β is released from platelets and immune cells, such as neutrophils. However, during the proliferation phase a lack of TGF-β activation might hinder re-epithelialization, angiogenesis and formation of the provisional ECM, as observed when all three TGF-beta isoforms are neutralized with neutralizing antibodies [76].

Biomaterial scaffolds mimicking ECM or incorporating ECM fragments has been proposed to enhance wound repair outcomes [77,78]. Therefore, an indirect implant coating by peptide bonds or mineral coating that allow timely release of the ECM1 [79,80], would be preferable to direct coating techniques, such as spray- or dip-coating. In contrast in the late remodeling phase application of ECM1 might be beneficial to prevent scar or keloid formation in respective risk patients. There the recombinant human ECM1 might be delivered later in the healing phase incorporated in hydrogel wound patches or as peptide-based nanoparticles [81–83]. However, in both cases presentation of ECM1 within the extracellular space needs to be provided to ensure its biological function.

5.2.1. Proposed Use of Tetrapeptide Sequences Targeting the Interaction of ECM1 with Proteases

The knowledge of specific motifs either in the ECM1 protein or the interacting proteases offers great perspectives for the modulation of the ECM1 effects. Much of this knowledge is obtained from murine studies on the effect of TGF-β signaling in liver fibrosis. On the one hand, hepatocyte specific knockout of ECM1 caused latent TGF-β1 activation and spontaneously induced liver fibrosis with rapid mortality [40,84]. On the other hand, overexpression of ECM1 was able to attenuate liver cirrhosis in mouse models [85,86]. In patients with chronic liver disease (CLD), ECM1 expression is inversely associated with the levels of TSP1, ADAMTS1, MMP-2, MMP-9, and LTGF-β1 activation [40,87].

Investigating the underlying effects, ECM1 was shown to suppress the activity of the proteases TSP1, ADAMTS1, MMP-2, and MMP-9, all involved in latent TGF-β1 activation. Immunoprecipitation experiments proved that ECM1 is binding directly to these enzymes. In case of TSP1 and ADAMTS1, specific tetrapeptide sequences were identified that control this interaction – the KRFK and KTRF motifs [40]. Both motifs are proposed to induce a conformational change in the LTGF-β1 molecule, resulting in the release of the active TGF-β1 ligand without the need for additional proteolysis. In line with this, application of KRFK tetrapeptides, which is characteristic for the interaction with TSP1, induced activation of latent TGF-β. This effect was partly attenuated by overexpression of ECM1 [40]. In contrast, application of KTRF tetrapeptides suppressed activation of latent TGF-β, even in ECM1 knock-out mice [40].

The knowledge of this opposite effect of these two tetrapeptides offers great perspectives for the biofunctionalization of implants. Especially, as due to their very small size, the tetrapeptides can be applied to the implants in larger amounts than the recombinant human ECM1 by using the same application methods described before. As the KTRF tetrapeptide simulates the ECM1 effect, its application might be beneficial later in the healing cascade when a resolution of the inflammation is required to prevent excessive scar formation. The KRFK tetrapeptides, that were shown to antagonize the ECM1 effects, might exert beneficial effects early in the proliferation phase, such as improves re-epithelialization, angiogenesis and ECM production.

5.2.1. Proposed Use of RNA and DNA-based Methods to Regulate ECM1 Expression

Using RNA or DNA-based methods might have the advantage that the target gene needs to be expressed and secreted by the local cells, which causes a natural delay in the proposed effects. This is of advantage when ECM1 shall be overexpressed, as this is supposed to interfere with the early phases of wound healing. Given this required delay of the proposed effect, this argues for DNA-based rather than RNA-based overexpression of ECM1 – preferably delivered as episomes or plasmids that allow transient expression of the transferred gene without integration into the genome.

In contrast, suppression of ECM1 might be beneficial in the early proliferation phase of the healing cascade, where active TGF-β supports re-epithelialization, angiogenesis and ECM production. There a timelier effect is required, which argues for a RNA-based rather than DNA-based knock-down of ECM1. However, similar to the DNA-based methods, the delivery technique mas have a significant effect on the speed and duration of the response. Usually, lnRNAs and aptamers provide the most rapid response with the shortest duration of the effect, lasting between hours and days. This is due to their short half-life, which for lnRNAs is defined mainly by their primary and secondary structure, but also by their interaction with other proteins within the cellular context [88]. The half-life of aptamers, in contrast, is defined by renal clearance and nuclease degradation. However, their half-life and therapeutic effect can be extended through modifications such as PEGylation, which increases their size and resistance to nucleases [89]. An intermediate effect duration (~5 days) is expected from miRNAs, which have an average half-life of about 5 days. Their stability is enhanced when forming a complex with the argonaute proteins as part of the RNA-induced silencing complex (RISC) [90]. Synthetic miRNAs follow the same degradation kinetics as siRNAs, thus the expected effect duration is also comparable (5 to 7 days). A more persistent effect is obtained by shRNAs that are often expressed from a plasmid or viral vector by the host cells. Ths

might be even permanent, when the delivery method includes integration into the hosts' genome [91], however, this is unfavorable for therapeutic use.

6. Conclusion

Wound healing depends on tightly regulated TGF-β pathway activity, orchestrated by ECM components, proteases, and receptor modulators. ECM1 emerges as a critical stabilizer of this system, ensuring balanced growth factor availability and matrix organization. As a protein predominantly localized within the ECM, ECM1 is a highly promising candidate for the biofunctionalization of implants due to its principal actions within the extracellular space. In light of its proposed mechanisms, several approaches for biofunctionalization are feasible: Direct strategies include the application of rh-ECM1 or the tetrapeptides KTFR and KFRK. Indirect strategies may utilize RNA- or DNA-based techniques—such as siRNAs, shRNAs, aptamers, messenger RNA, or plasmids—to achieve knockdown or overexpression of ECM1. These molecules can be delivered to implants either alone, with the aid of polymers or vesicles, or incorporated into hydrogels.

Depending on the selected strategy, the delivered protein/peptide, RNA, or DNA may either activate or inhibit the proteases ADAMTS1, MMP2, and MMP9, as well as TSP1, which are involved in the proteolytic release of active TGF-β from its latent complex. Ultimately, leveraging the modulatory capacity of ECM1 in wound and fracture healing—via its regulation of TGF-β signaling—holds significant promise for addressing the substantial clinical challenges posed by non-unions, impaired wound healing, and chronic wounds.

Abbreviations

The following abbreviations are used in this manuscript:

ADAMTS1	a disintegrin and metalloproteinase with thrombospondin motifs 1
Alk	activin receptor-like kinase
CLD	chronic liver disease
DOAJ	Directory of open access journals
ECM	extracellular matrix
BAMBI	BMP and Activin Membrane-Bound Inhibitor
ECM1	Extracellular matrix protein-1
HTRA1	high temperature requirement factor A1
KRFK	lysine-phenylalanine-arginine-lysine
KTRF	lysine-tryptophan-arginine-phenylalanine
LAP	latency-associated peptide
LD	Linear dichroism
LLC	large latent complex

LTBP	latent TGF-β binding protein
LTGF-β1	latent TGF-β1
MDPI	Multidisciplinary Digital Publishing Institute
MMP	matrix metalloproteinases
RGD	arginine-glycine-aspartic acid
shRNAs	small hairpin RNAs
siRNAs	small interfering RNAs
Smad	small mothers against decapentaplegic
TGF-β	transforming growth factor-beta
TLA	Three letter acronym
TSP1	thrombospondin 1
VEGF	vascular endothelial growth factor

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