

## Principles and mechanisms of peri-implant soft tissue healing

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The long-term clinical and esthetic success of implant-supported restorations is determined by osseointegration and optimal remodeling of peri-implant soft tissues. Complications of soft-tissue management are often caused by fibrotic regeneration of oral mucosa after multiple surgical procedures. Knowledge of the proliferative processes in wound healing is necessary to attain adequate soft-tissue conditions. Successful reconstruction of peri-implant soft tissues is feasible even in fibrotic conditions when appropriate surgical techniques are selected. The pleiotropic proliferative cytokine TGF- $\beta$  is involved in the regulation of all phases of wound healing and tissue remodeling. The isoform TGF- $\beta_1$  is a cytokine associated with the development of fibrotic tissue. Overexpression of TGF- $\beta_1$  causes scarring and fibrosis, and results in limited clinical success of intraoral soft-tissue management. Experimental therapeutic approaches with neutralizing antibodies to block TGF- $\beta_1$  resulted in less scarring and a reduction of fibrosis. Further molecular biologic research of cell-matrix-cytokine interactions in wound healing will provide highly specific antifibrotic therapeutic approaches in the future. (*Quintessence Int* 2005;36:759–769)

**Key words:** fibrosis, implants, soft tissue, transforming growth factor-beta, wound healing

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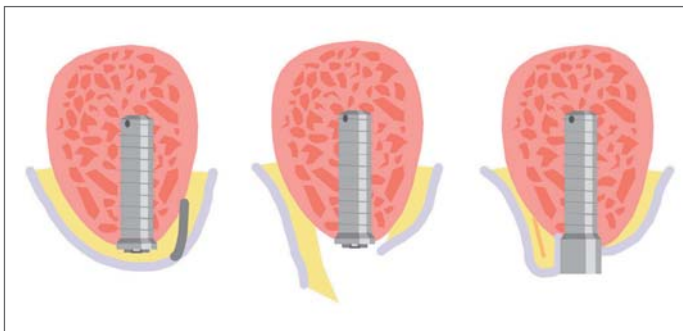
The long-term clinical and esthetic success of an implant-retained restoration is determined by stable peri-implant soft-tissue morphology in harmony with the surrounding soft tissues and natural dentition. In addition to successful osseointegration of the implant, the surrounding soft tissues play an important role in vascularization of the bone.<sup>1,2</sup> Insufficient peri-implant tissues may cause a nutritive undersupply of the bone resulting in implant loss due to resorption.<sup>3</sup> Proper gingival architecture is especially important in relation to anterior esthetics.<sup>4</sup> Thorough treatment planning and knowledge of the specific phases of inflammatory and regenerative processes associated with wound healing are essential for predictable results. Preoperative soft-tissue deficiencies often require extensive mobilization and dis-



**Fig 1** Buccal view of implant restoration with hard- and soft-tissue deficiencies and peri-implant inflammation.



**Fig 2** Buccal view of peri-implant scar tissue.



**Fig 3** Schematic drawing of the roll-flap technique.

placement of existing tissues. Several medical factors such as diabetes, hypertension, surgical pretreatment, and radiation therapy influence wound healing after soft tissue maturation and may lead to necrosis, fibrotic scar tissue, deficient soft-tissue architecture, and unacceptable esthetic results (Figs 1 and 2).<sup>5</sup>

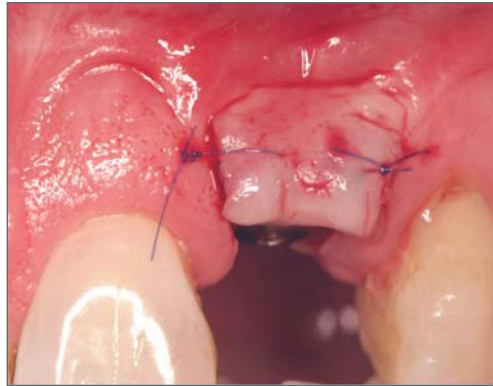
Several surgical techniques may be applied to obtain an adequate emergence profile of the restoration with sufficient keratinized gingiva<sup>6,7</sup>:

1. To maintain an adequate amount of keratinized gingiva:
  - Crestal incision
2. For local transposition of keratinized gingiva:
  - Vestibular-oral transposition
  - Pedicle graft/roll flap techniques
  - Split thickness flaps

3. To reconstruct new keratinized gingiva:
  - Free soft-tissue grafts in combination with vestibuloplasty
4. Future techniques (tissue engineering):
  - Transplantation of autologous keratinocytes cultivated in vitro, in combination with vestibuloplasty

Improvement of the clinical situation and esthetics of the patient shown in Fig 2 was achieved with a roll flap technique for closure of the defect (Fig 3). The advantage of this technique is the perfect blending with the surrounding tissues (Fig 4).

Satisfactory results may be obtained when inflammatory and proliferative processes are accounted for and adequate surgical techniques are employed (Figs 5 and 6). Thorough understanding of physiologic and potentially deficient soft-tissue morphology, as well as



**Figs 4a and 4b** Application of the roll-flap technique.



**Figs 4c and 4d** An adequate final result could be obtained despite the severe soft-tissue deficiency.



**Figs 5a and 5b** The peri-implant gingival architecture was reconstructed appropriately.



**Figs 6a and 6b** Buccal view prior to and after implant placement.

regulatory and pathologic mechanisms on a cellular basis is important in complex cases.

## WOUND HEALING

### Morphology and physiology of intraoral wound healing

Complex biological interactions on a cellular and biochemical level follow every soft-tissue management procedure. These cascades are initiated with second-stage surgery and end after soft-tissue integrity has been re-established. Bone is the only tissue with the competency to heal unscarred. Healing of any other organ leads to formation of regenerated fibrotic tissues. After peri-implant surgery, wound healing proceeds in 3 phases which are partially superimposed: (1) inflammatory phase, (2) proliferative phase, and (3) remodeling phase.<sup>8-10</sup>

The inflammatory phase initiates wound healing through hemostasis, coagulation, increased vascular permeability for specialized cells, and chemotaxis. The release of inflammatory mediators and growth factors activates neutrophilic granulocytes and macrophages. After second-stage surgery/soft-tissue augmentation and resultant vasoconstriction, coagulation and complement cascades are activated. Thrombocytes degranulate inside the lesion. Coagulation and activation of thrombocytes limit blood loss. Monocytes infiltrate the site and differentiate to macrophages. In addition to phagocytosis of detritus, cytokine-activated macrophages secrete

angiogenic and fibroblast-stimulating cytokines. A provisional fibrinogenous wound closure completes the first phase.<sup>10,11</sup>

Formation of granulation tissue and epithelium dominates the proliferative phase. Mobile fibroblasts, stimulated by cytokines, migrate into the lesion. Proliferated fibroblasts secrete extracellular matrix components, primarily fibrin, fibronectin, glycosaminoglycans, and collagen. Surface receptors of adhesion molecules such as tenascin and laminin mediate the composition of a new matrix.<sup>8,10,14,15</sup> The morphology of keratinocytes changes a few minutes after soft tissue surgery. Following infringement of oral soft tissue, the multilayered epithelium thickens, and basal cells enlarge and migrate into the lesion to close the defective area. Subsurface migration separates necrotic tissues from the lesion. Three days after a trauma, regenerated collagen fibers are traceable and new capillaries migrate from intact venules into the wound.<sup>8,15-17</sup>

The third and final phase of wound healing overlaps the proliferative phase. Remodeling of the provisional connective tissue matrix forms mucoperiosteal matrix. Collagen deposition in the wound continues, changing the collagen and matrix compositions. Fibroblastic integrin-receptor patterns initiate other cell-matrix interactions. Cytokines regulate integrin-receptor expression.<sup>8</sup> This is the most important phase of wound healing from a clinical point of view, since the quality of the regenerated tissue is determined at this stage. Collagen types I and III are the dominating forms in the skin and mucosa. The degree of

<b>Table 1 Key cytokines for soft tissue healing, their respective target cells, and biologic response</b>			
<b>Cytokine</b>	<b>Cellular depot</b>	<b>Target cell</b>	<b>Biologic activity</b>
TGF-β <sub>1</sub> , TGF-β <sub>2</sub>	Macrophages, thrombocytes, fibroblasts, keratinocytes	Leukocytes, keratinocytes, fibroblasts	Chemotaxis, proliferation, matrix production (fibrosis)
TGF-β <sub>3</sub>	Macrophages	Fibroblasts	Anti-scarring
TGF-α	Macrophages, thrombocytes, keratinocytes	Keratinocytes, fibroblasts, endothelial cells	Proliferation
TNF-α	Neutrophiles	Macrophages, keratinocytes	Activation of cytokines
PDGF	Macrophages, thrombocytes, fibroblasts, endothelial cells, vascular smooth muscle cells	Neutrophiles, macrophages, fibroblasts, endothelial cells, vascular smooth muscle cells	Chemotaxis, proliferation, matrix synthesis
FGF-1, FGF-2, FGF-4	Macrophages, fibroblasts, endothelial cells,	Keratinocytes, fibroblasts, endothelial cells, chondrocytes	Angiogenesis, proliferation, chemotaxis
FGF-7 (KGF)	Fibroblasts	Keratinocytes	Proliferation, chemotaxis
EGF	Thrombocytes, macrophages, keratinocytes	Keratinocytes, fibroblasts, endothelial cells	Proliferation, chemotaxis
IGF-1/Sm-C	Fibroblasts, macrophages	Fibroblasts, endothelial cells	Proliferation, collagen synthesis
IL-1α and IL-1β	Macrophages, neutrophiles	Macrophages, fibroblasts, keratinocytes	Proliferation, collagenase synthesis, chemotaxis
CTGF	Fibroblasts, endothelial cells	Fibroblasts	Amplification of TGF-β <sub>1</sub>
VEGF	Macrophages, keratinocytes	Endothelial cells	Angiogenesis

continuous long-term cicatricial contracture is a determining factor for peri-implant soft tissue architecture.

**Regulation of wound healing**

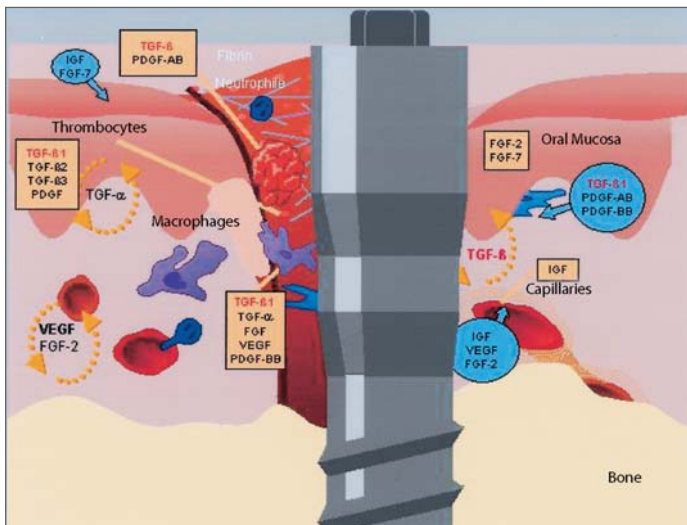
Biologic healing following peri-implant soft-tissue surgery is controlled by cytokines (polypeptide messengers). In addition to well-known hormone regulatory mechanisms, cytokines also have paracrine, autocrine, and multifunctional qualities. Cell responses are mediated by specific surface receptors.<sup>11</sup>

Various cytokines arrange primary cell activation in the inflammatory phase of peri-implant wound healing. Interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), insulin-like growth factor-1, and transforming growth factor-beta (TGF-β) are predominantly involved.<sup>18</sup> Cytokine expression from injured vascular endothelium and degranulated thrombocytes affects neutrophile granulocytes and macrophages through chemotaxis.<sup>10</sup> Platelet-derived growth factor (PDGF) is also released from the thrombocytes and has a chemotactic effect on macrophages, neutrophile granulocytes,

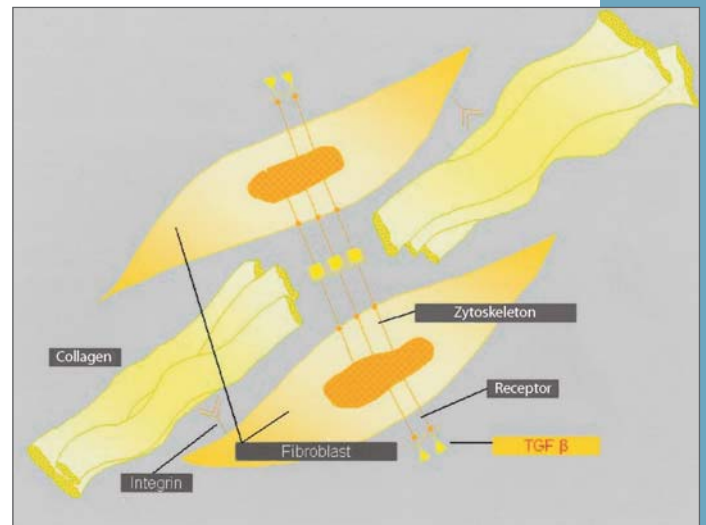
endothelial cells, and fibroblasts. It is a potent mitogenic cytokine that stimulates production of noncollagenous matrix components.<sup>19,20</sup> The epithelium cell migration and sufficient growth of new capillaries are crucial for wound closure and reconnection of detached tissues with blood circulation. Animal studies and clinical trials have shown that angiogenesis begins on the third day of healing. New capillaries supply the regenerated tissues on the seventh day.<sup>21,22</sup> Angiogenesis is induced by vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF<sub>2</sub>). Tissue hypoxia is the strongest stimulus for release of VEGF. VEGF and FGF<sub>2</sub> activate proliferation of endothelial cells and boost capillary growth.<sup>23</sup>

TGF-β occupies a central position in every phase of wound healing. It is a multifunctional signal protein. Numerous research groups demonstrated that both overexpression and lack of TGF-β may be responsible for an impaired healing process. Cytokines involved in healing mechanisms are displayed in Table 1. Cellular communication and complex cell-cytokine-matrix interactions are necessary for undisturbed implant integration.<sup>24,25</sup>





**Fig 7** Schematic drawing of the complex inflammatory and proliferative processes in peri-implant tissues.



**Fig 8** Regulation of soft-tissue healing through cell-matrix interaction.

### TGF-β and healing

TGF-β is excreted from thrombocytes, macrophages, and fibroblasts (Fig 7). It attracts other fibroblasts, monocytes, and macrophages to the site of inflammation.<sup>26–28</sup> During new tissue formation, it induces the expression of integrins, which control the migration of keratinocytes to the wound surface.<sup>29</sup> Additionally, TGF-β stimulates the synthesis of collagen (predominantly collagen I and III) during the wound healing process.<sup>30–33</sup> In the course of remodeling, the cytokine is believed to influence the composition and cross-linking of permanent collagen structures by regulating the pattern of integrin expression in fibroblasts<sup>30,34–37</sup> (Fig 8).

TGF-β itself is regulated during physiologic wound healing. Tissue concentrations vary throughout the healing process, with a maximum expression in the initial phase.<sup>38</sup>

The impact of TGF-β on angiogenesis is unclear. Studies showed that, depending on its concentration, TGF-β either stimulates angiogenesis, or inhibits the process if present in high concentrations.<sup>39,40</sup> Experimental research also showed varying results when in vivo and in vitro results were compared. An angiogenic effect was verified in vitro,<sup>40,41</sup> while the angiogenesis promotion of TGF-β is doubted by some authors in in vivo settings.<sup>42</sup>

### Structure, synthesis, and regulation of TGF-β

TGF-β forms a TGF-β superfamily of polypeptide growth factors along with bone morphogenetic proteins (BMPs) and activins.<sup>43</sup> TGF-β is a highly preserved cytokine with 3 known isoforms of structural resemblance in mammals.<sup>43,44</sup>

TGF-β<sub>1</sub> is expressed from endothelial, hematopoietic, and connective tissue cells; TGF-β<sub>2</sub> from epithelial and neuronal cells; and TGF-β<sub>3</sub> predominantly from mesenchymal cells.<sup>43</sup> These proteins are biologically inactive homodimer precursor proteins, which are split into a C-terminal and N-terminal component.<sup>45</sup> The C-terminal TGF-β remains in a biologically inactive form with noncovalent binding to the N-terminal part of latency Associated Peptide (LAP). TGF-β and LAP are both disulfate-connected homodimers,<sup>46</sup> LAP is usually bound to latent TGF-β binding protein (LTBP), which mediates cellular or matrix contacts.<sup>47,48</sup>

TGF-β becomes active by dissociation of LAP.<sup>45</sup> Triggering factors of a physiologic activation of TGF-β currently are not well understood.<sup>49</sup> The activation of TGF-β is initiated by plasmin,<sup>50</sup> thrombospondin-1,<sup>51</sup> ionizing radiation energy,<sup>52–54</sup> in vitro heat (100°C),<sup>52,53</sup> and extreme pH changes (pH 2 or pH 8).<sup>55</sup>

Three groups of TGF- $\beta$  receptors are known: TGF- $\beta$ R-I, TGF- $\beta$ R-II, and TGF- $\beta$ R-III.<sup>56-58</sup> TGF- $\beta$ R-III (betaglycane-receptor) is most notably expressed. It can be traced to fetal and adult tissues of mesenchymal, epithelial, and neuronal origin.<sup>59</sup> TGF- $\beta$ R-III binds to all 3 TGF- $\beta$  isoforms, but has the greatest affinity to TGF- $\beta_2$ .<sup>57,60,61</sup> Being a membranous receptor, TGF- $\beta$ R-III enhances the affinity of TGF- $\beta$  to TGF- $\beta$ R-I and TGF- $\beta$ R-II without being involved in signal transduction processes.<sup>62</sup> TGF- $\beta$ R-I and TGF- $\beta$ R-II mediate the signal transduction. Both are transmembranous receptors with serine/threonine kinase activity on the inner side of a cell. On the surface they can be present as TGF- $\beta$ R-I-homodimers, TGF- $\beta$ R-II-homodimers, and TGF- $\beta$ R-I/II-heterodimers.<sup>63</sup> Binding of TGF- $\beta$  to TGF- $\beta$ R-II leads to the formation of a TGF- $\beta$ R-II/R-I heteromer on the extracellular side of the membrane.

In absence of TGF- $\beta$ R-I, TGF- $\beta$ R-II binds to TGF- $\beta_{1+3}$  with a high affinity, but not to TGF- $\beta_2$ .<sup>64</sup> Coexpression of TGF- $\beta$ R-I and TGF- $\beta$ R-II leads to an efficient binding of TGF- $\beta_2$ .<sup>65</sup> In absence of TGF- $\beta$ R-II, TGF- $\beta$ R-I does not bind any TGF- $\beta$  isoform efficiently. TGF- $\beta$ R-II binds to all TGF- $\beta$  isoforms.<sup>59</sup> After TGF- $\beta$  binds to TGF- $\beta$ R-II and TGF- $\beta$ R-I/II, heterodimer formation activates TGF- $\beta$ R-II kinase by phosphorylation of TGF- $\beta$ R-I.<sup>66</sup> The TGF- $\beta$ R-I kinase phosphorylates and activates carriers of the intracellular signal transduction, the so-called Smads.<sup>67</sup>

Smad proteins are a family of transcription factors transferring signals of activated receptor complexes from the cytoplasm to the nucleus. One common characteristic is highly preserved protein structures.<sup>68</sup> Smads are subdivided in 3 groups: receptor-regulated smads (R-Smads), common mediator Smads (Co-Smads), and inhibitory Smads (I-Smads).<sup>69</sup>

The receptor kinase of TGF- $\beta$ R-I (activated by binding to TGF- $\beta$ ) phosphorylates specifically Smad2/3 protease from the receptor-associated Smads. A regulatory complex for the transcription originates in the nucleus through cooperative binding with Smad4 (Co-Smad).<sup>70</sup>

## TGF- $\beta$ AND IMPAIRED HEALING

Both the lack and overexpression of TGF- $\beta$  may be responsible for an impaired healing process. TGF- $\beta$  stimulates the deposition of collagen and matrix components through fibroblasts. At the same time, it inhibits collagenase and plasminogen activators. High tissue concentration of TGF- $\beta$  hinders matrix degradation and causes an increased collagen deposition.<sup>71</sup> Changes of the integrin level of collagen-producing fibroblasts result in atypical composition of collagens and leads to an abnormal cross-linking.<sup>72</sup> The fact that increased levels of TGF- $\beta$  were found in fibrotic gingival tissues and keloids emphasizes the role of TGF- $\beta$  as a promoter for fibrosis.<sup>71,73-77</sup>

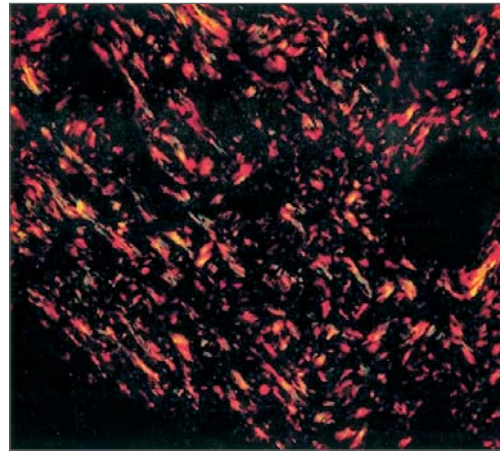
Skin tissue *in vitro* has shown fibrotic conversion and increased synthesis of fibronectin and tenascin when increased levels of TGF- $\beta$  were present for a prolonged amount of time.<sup>78</sup> Subcutaneous application of TGF- $\beta$  induced fibrosis in animal studies.<sup>79</sup> In a comparison study of adult and fetal tissues, the reduced level of TGF- $\beta$  in fetal tissue was shown to prevent scarring.<sup>80</sup>

The role of TGF- $\beta$  in mediating impaired soft tissue healing is of great importance in implantology in terms of both atrophic and scarred tissues.

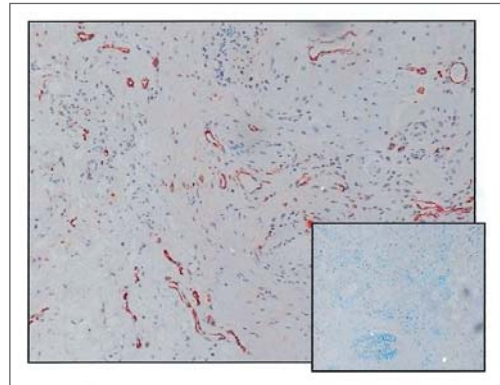
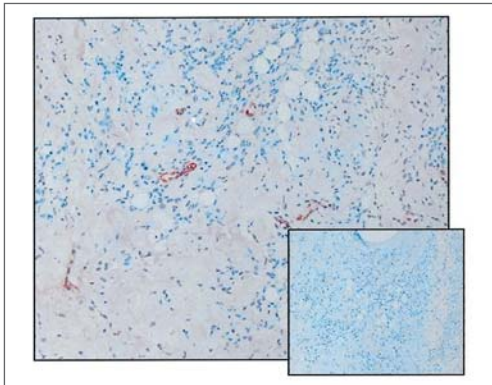
During the inflammatory phase, overexpression of TGF- $\beta$  causes reduced phagocytosis capability of granulocytes and macrophages, which leads to delayed wound healing and increased risk of infection.<sup>81</sup> Research results showed a reduction of fibrotic processes and endothelium proliferation, as well as less wound contraction.<sup>82</sup>

Formation of granulation tissues and mucous epithelium are delayed during the proliferative and remodeling phase.<sup>83</sup> Clinical and experimental studies on soft tissue maturation indicated a delayed and quantitatively reduced capillary vascularization with a distinctive pericapillary fibrosis.<sup>22</sup> Evidence was provided for an augmented expression of collagen I, II, and IV.<sup>84</sup>

The present authors established a protocol for animal studies that demonstrates a significant increase in TGF- $\beta_1$ , LAP, and



**Figs 9a and 9b** Histology of scar tissue. (a) Connective scar tissue is visible after staining (Sirius-Red) and polarized microscopy. (b) Reduction of scar tissue was possible after applying neutralizing antibodies (anti-TGF- $\beta_1$ ).



**Figs 10a and 10b** Immunohistochemical marking/staining (CD105) of vascularization was improved after anti-TGF- $\beta_1$  therapy. Vascularization of scar tissue (a) is compared to vascularization after scar reducing antibody therapy (b).

TGF $\beta_2$  expression. Results were correlated to the severity of clinically impaired wound healing. Marginal areas of adapted and transplanted skin and mucosa are primarily involved.<sup>85-87</sup> In other animal studies, the postoperative, exogenous application of TGF- $\beta_1$ -neutralizing antibodies obtained a reduced TGF- $\beta_1$  expression and a reduction of scar tissue during healing (Fig 9). The degree of capillary and overall vascularization was increased through the therapeutic adoption of the antibody (Fig 10).

## CONCLUSION

The understanding of the cytokine-mediated processes of connective tissue formation improves the planning and execution of physiologic soft-tissue management while avoiding fibrosis-inducing techniques. Current research on the regulation of matrix synthesis and pathologic mechanisms of fibrosis indicates significant reduction of scarring. Future experimental and clinical trials are necessary to better understand the interaction of complex cytokine networks of wound healing on a cellular and molecular-biologic level. Such work will provide the basis for establishing highly specific and selective therapeutic approaches to reduce scarring and fibrosis.

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