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CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

Myofibroblasts in Palatal Wound Healing: Prospects for the Reduction of Wound Contraction after Cleft Palate Repair

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ABSTRACT

The surgical closure of orofacial clefts is considered to impair maxillary growth and dento-alveolar development. Wound contraction and subsequent scar tissue formation, during healing of these surgical wounds, contribute largely to these growth disturbances. The potential to minimize wound contraction and subsequent scarring by clinical interventions depends on the surgeon's knowledge of the events responsible for these phenomena. Fibroblasts initiate wound contraction, but proto-myofibroblasts and mature myofibroblasts are by far the most important cells in this process. Myofibroblasts are characterized by their cytoskeleton, which contains alpha-smooth-muscle actin. Additionally, their contractile apparatus contains bundles of actin microfilaments and associated contractile proteins, such as non-muscle myosin. This contractile apparatus is thought to be the major force-generating element involved in wound contraction. After closure of the wound, the myofibroblasts disappear by apoptosis, and a less cellular scar is formed. A reduction of contraction and scarring might be obtained by inhibition of myofibroblast differentiation, stimulation of their de-differentiation, stimulation of myofibroblast apoptosis, or impairment of myofibroblast function. In this review, we will discuss all of these possibilities, which ultimately may lead to a better outcome of cleft palate surgery.

KEY WORDS: cleft palate, wound healing, myofibroblasts, wound contraction.

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(1) INTRODUCTION

Orofacial clefts are defined as congenital defects in which the fusion between two or more of the following structures has failed: the palatal shelves, the maxillary prominences, and the medial nasal prominences. The clefts are surgically closed to restore the integrity of the oral and nasal cavity, and to allow for normal feeding and speech development. A modified Von Langenbeck method is often used for palatoplasty, the initial surgical repair of the palate (Ross, 1987). Incisions are made on both sides of the cleft and adjacent to the alveolar bone, and the mucoperiosteal flaps are mobilized and sutured together in the midline. As a consequence, this technique results in lateral open wounds with denuded bone. Healing of these wounds is associated with the disadvantageous effects on maxillary growth and dento-alveolar development often seen in cleft palate patients. Many alternative methods for palatoplasty have been developed, but growth disturbances still occur (Millard, 1976). Two different but related wound-healing events—namely, wound contraction and scar tissue formation—contribute largely to these undesirable consequences (Kremenak, 1984; Wijdeveld *et al.*, 1987a, 1991). Wound contraction reduces the size of the defect, but it also induces substantial scarring, ultimately resulting in the disadvantageous effects on growth (Wijdeveld *et al.*, 1987b, 1991). A reduction of wound contraction and subsequent scarring is therefore desirable.

Fibroblasts play an important role in both processes (Grinnell, 1994; Clark, 1996; Badid *et al.*, 2000). They display a considerable degree of inter- and intra-site heterogeneity in phenotype and activity (Irwin *et al.*, 1994; Lekic *et al.*, 1997). Differences in migration, integrin expression, cell proliferation, and collagen synthesis by fibroblasts obtained from different phases of wound healing have been reported (Finesmith *et al.*, 1990; Fries *et al.*, 1994; Irwin *et al.*, 1994; Badid *et al.*, 2000; Van Beurden *et al.*, 2003). A specialized fibroblast type, involved in wound contraction, is the myofibroblast. Its major characteristic is the expression of alpha-smooth-muscle actin (α-SM actin). Myofibroblasts are present in organs with a high remodeling capacity, such as the kidneys, the lungs, and the periodontal ligament (Gabbiani, 1994, 1998; Desmouliere and Gabbiani, 1996; Lorena *et al.*, 2002; Tomasek *et al.*, 2002), or during increased remodeling, such as in growth, development, inflammatory responses, and the contraction of healing wounds (Squier and Kremenak, 1980; Gabbiani, 1992). In contrast, hardly any myofibroblasts are present in tissues with a low remodeling activity, as in normal dermis (Squier and Kremenak, 1980; Cornelissen *et al.*, 2000b; Van Beurden *et al.*, 2003).

Myofibroblasts were first described in 1971 (Gabbiani *et al.*, 1971). Since then, the role of myofibroblasts has been intensively studied (Desmouliere, 1995; Desmouliere and Gabbiani, 1996; Powell *et al.*, 1999; Moulin *et al.*, 2000; Hinz *et al.*, 2001; Van Beurden *et al.*, 2003). Myofibroblasts cause the extracellular matrix to contract (Clark, 1996) and are involved in the regulation of proliferation and differentiation of epithelial, vascular, and neurogenic cells (Saunders and D'Amore, 1992; Yamagishi *et al.*,

1993). This review will focus on the role of myofibroblasts in wound contraction during wound healing after palatoplasty. The factors involved in the differentiation of fibroblasts into myofibroblasts will be emphasized. Understanding the biology of these factors might be a starting point for the development of strategies to reduce undesired contraction and subsequent scarring.

(2) WOUND HEALING

Wound healing can be divided into three subsequent, partly overlapping, phases, namely, inflammation, proliferation, and tissue remodeling (Clark, 1996). Following injury, vasoconstriction reduces hemorrhage and favors platelet aggregation. Almost concurrently, vasodilatation enables inflammatory cells to enter the site of injury and clean the wound. Neutrophils and macrophages migrate into the wound to prevent the invasion and proliferation of micro-organisms (Ehrlich and Krummel, 1996). Platelet aggregation and coagulation result in the formation of a provisional fibrin clot that covers the wound (Clark, 1996; Ehrlich and Krummel, 1996).

Second, the proliferation phase starts with the migration of fibroblasts into the wound area and their propagation. These fibroblasts start to produce granulation tissue components, such as fibronectin, collagen, and hyaluronic acid (Clark, 1996). Some fibroblasts differentiate into myofibroblasts, which are principally responsible for tissue contraction, but also produce extracellular matrix components (Sappino *et al.*, 1990; Grinnell, 1994; Ehrlich and Krummel, 1996). In skin wounds, myofibroblasts are abundantly present up to two weeks post-wounding (Greenhalgh, 1998; Huang *et al.*, 2003). Simultaneously, re-epithelialization occurs by proliferation and migration of epithelial cells from the wound edges. Soon after re-epithelialization, wound contraction stops, and myofibroblasts start to disappear, probably through apoptosis (Desmouliere *et al.*, 1995; Clark, 1996; Huang *et al.*, 2003).

Third, during the remodeling phase, the number of blood vessels declines, and apoptosis of fibroblasts results in scar tissue with a low cell density (Clark, 1996). Ultimately, the scar contains only a few fibroblasts with a well-developed rough endoplasmic reticulum (Gabbiani, 1994). In pathological situations, such as hypertrophic scars, myofibroblasts may persist (Sappino *et al.*, 1990; Ehrlich *et al.*, 1994; Spyrou and Naylor, 2002).

Unlike adult skin, early fetal skin heals without wound contraction and scar tissue formation (Moulin *et al.*, 2001). A low ratio of transforming growth factor (TGF)β1,2 to TGFβ3 seems to cause this phenomenon (Moulin *et al.*, 2001). Interestingly, mice lacking the TGFβ1 antagonist TGFβ3 develop a cleft palate (Nawshad *et al.*, 2004). Furthermore, myofibroblasts hardly occur in early fetal wounds (McCluskey and Martin, 1995), while wounds of later gestational age show a temporary increase in the number of myofibroblasts (Schor *et al.*, 1996). Fetal wounds are also characterized by the absence of clot formation and inflammatory reactions. Taken together, at an early gestational age, the adult wound-healing process is not yet fully developed, which precludes wound contraction and scar tissue formation. At a later gestational age, these specific features of early fetal wounds disappear.

(2.1) Dermal *vs.* Oral Wound Healing

A general observation is that wounds in the oral mucosa heal faster and with less scarring than do dermal wounds (Hakkinen *et al.*, 2000), although the opposite is also claimed (Nooh and Graves, 2003). The cause of this difference is not completely understood, but saliva, leukocytes, growth factors, and specific fibroblast subpopulations seem to be involved (Noguchi *et al.*, 1991; Hormia *et al.*, 1993; Varshney *et al.*, 1997; Taichman *et al.*, 1998; Stephens *et al.*, 2001; Lepekhin *et al.*, 2002).

Saliva provides a unique environment favoring wound healing (Hakkinen *et al.*, 2000). Saliva-treated cutaneous wounds have a reduced inflammatory reaction, faster epithelial coverage, and a faster connective tissue regeneration (Zelles *et al.*, 1995; Varshney *et al.*, 1997; Kagami *et al.*, 2000; Ohshima *et al.*, 2002). Desalivated oral wounds contain fewer myofibroblasts than do saliva-treated wounds, and a delayed peak in their number (Bodner and Dayan, 1995). As a result, wound contraction and granulation tissue formation are delayed in desalivated rats (Hutson *et al.*, 1979; Bodner *et al.*, 1991). It appears that moisture and ionic strength are not the primary factors promoting wound healing (Zelles *et al.*, 1995). More likely, growth factors in saliva—such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF)—enhance tissue repair (Skaleric *et al.*, 1997; Taichman *et al.*, 1998; Fujisawa *et al.*, 2003). Lower levels of macrophages, neutrophils, and T-cells are present in oral wounds than in skin wounds (Szpaderska *et al.*, 2003). Furthermore, oral wounds contain fewer pro-inflammatory factors such as TGFβ1 and interleukin-6 (IL-6) (Bodner and Dayan, 1995; Hakkinen *et al.*, 2000; Szpaderska *et al.*, 2003). *In vitro* studies have shown that oral fibroblasts possess a higher capability to cause a collagen lattice to contract than do dermal fibroblasts (Stephens *et al.*, 1996, 2001; Sukotjo *et al.*, 2003). In the same *in vitro* model, oral fibroblasts produce higher levels of matrix metalloproteinase-2 (MMP-2) and reduced levels of tissue inhibitors of metalloproteinases (TIMPs) (Stephens *et al.*, 2001).

The fibroblast phenotype differs substantially between anatomical sites (Castor *et al.*, 1962; Moulin *et al.*, 1998; Lee and Eun, 1999; Chipev and Simon, 2002). Different fibroblast subpopulations are also present in oral and dermal tissues. Intra-oral fibroblasts generally exhibit a more fetal-like phenotype with a remodeling capacity higher than that of dermal fibroblasts (Sloan, 1991; Irwin *et al.*, 1994; Stephens *et al.*, 2001). Dermal and oral fibroblasts secrete different types and amounts of glycosaminoglycans (GAGs) (Bronson *et al.*, 1988). Also, differences in migrational behavior (Lepekhin *et al.*, 2002), adhesional properties (Palaiologou *et al.*, 2001), expression of extracellular matrix receptors (Palaiologou *et al.*, 2001), and response to growth factors like TGFβ1 (Lee and Eun, 1999) have been reported. However, all the above findings are from studies on fibroblasts derived from buccal, periodontal, or gingival wounds. Studies on palatal fibroblasts are lacking. In the palate, specific structural features give rise to a different outcome of the wound-healing process. The palatal soft tissue is a rigid mucoperiosteum that is attached to the palatal bone. The healing of open wounds on the palate after cleft palate closure results in an additional periosteal osteogenic reaction, which tightly anchors the scar tissue to the palatal bone. This immobile scar impairs maxillary growth and development of the dentition after palatoplasty (Wijdeveld *et al.*, 1991).

Thus, oral wound healing differs from dermal wound healing in several respects. The faster wound-healing rate and the decreased scar formation indicate a more fetal type of wound healing. These differences are caused by the local environment and specific fibroblast subpopulations. In spite of these favorable characteristics, the specific features of palatal wound healing are responsible for the growth disturbances observed after cleft palate repair.

(2.2) Wound Contraction

The first concept of wound contraction was postulated in 1971 (Gabbiani *et al.*, 1971). It was based on the involvement of myofibroblasts. Wound contraction is achieved by the concerted action of many myofibroblasts (Desmouliere and Gabbiani, 1996), and is mediated by many cell-cell and cell-matrix contacts, resulting in a re-arrangement or shortening of the collagen fibrils (Welch *et al.*, 1990). The second concept (Ehrlich and Rajaratnam, 1990) states that the locomotion of normal fibroblasts leads to the contraction of a wound. It states that fibroblasts do not act in a coordinated manner, but that the tractional forces of many individual fibroblasts are responsible for wound contraction.

Recently, a combination of these two concepts has been advocated by Tomasek *et al.* (2002), who state that migrating fibroblasts initially exert tractional forces on the surrounding collagen matrix. This mechanical tension stimulates fibroblasts to differentiate into proto-myofibroblasts by the development of stress fibers (Tomasek *et al.*, 2002). Fibroblasts under tension *via* the extracellular matrix also express TGFβ1 (Varedi *et al.*, 1997). Proto-myofibroblasts generate contractile forces without the expression of α-SM actin. They are also able to synthesize and organize fibronectin, and to form small fibronexi. Proto-myofibroblasts can differentiate into mature myofibroblasts in response to specific factors, like TGFβ1, ED-A fibronectin (ED-A FN), and mechanical tension (Tomasek *et al.*, 2002). In the cornea, keratinocytes seem to differentiate directly into myofibroblasts (Jester *et al.*, 2002). Mature myofibroblasts express α-SM actin in elaborate stress fibers, and form large fibronexi. These differentiated myofibroblasts are present in late-contracting granulation tissue (Tomasek *et al.*, 2002). After closure of the wound, mature myofibroblasts disappear through apoptosis (Desmouliere *et al.*, 1995; Funato *et al.*, 1999; Moulin *et al.*, 2000) or by de-differentiation (Desmouliere, 1995).

(3) DEFINITION, ORIGIN, AND CHARACTERIZATION OF THE MYOFIBROBLAST

The simplest definition of a myofibroblast is that it is a fibroblast with smooth-muscle cell-like features (Powell *et al.*, 1999). Although the characteristics of myofibroblasts have been well-described, there is no consensus about their origin. Based on morphological observations, it has been postulated that myofibroblasts are derived from smooth-muscle cells (Fisher *et al.*, 1978; Sottiurai *et al.*, 1978; Shum and McFarlane, 1988). However, this concept was rejected on the basis of immunocytochemical studies (Schurch *et al.*, 1984; Eddy *et al.*, 1988). Other concepts state that myofibroblasts develop directly from mesenchymal cells (Dominguez-Malagon, 1993), epithelial cells (Bariety *et al.*, 2003), pericytes (Lindahl *et al.*, 1997), or circulating fibrocytes (Abe *et al.*, 2001; Metz, 2003). The most widely accepted concept is that myofibroblasts originate from fibroblasts (Gabbiani *et al.*, 1971, 1976; Gabbiani and Badonnel, 1976; Gokel and Hubner, 1977; Ariyan *et al.*, 1978; Grinnell, 1994; Masur *et al.*, 1996; Gabbiani, 2003). In healing wounds, an influx of fibroblasts from the surrounding tissue is observed. These fibroblasts differentiate into myofibroblasts after the appropriate stimuli (Schmitt-Graff *et al.*, 1994).

Myofibroblasts are characterized by their cytoskeleton, which contains α-SM actin, an actin isoform also present in smooth-muscle cells (Darby *et al.*, 1990; Desmouliere and Gabbiani, 1996; Gabbiani, 1998, 2003; Tomasek *et al.*, 2002). Additionally, their contractile apparatus contains bundles of actin microfilaments and associated contractile proteins, such as non-muscle myosin. This contractile apparatus is the major force-generating element involved in wound contraction (Desmouliere, 1995), and it is also found in cultured fibroblasts (Powell *et al.*, 1999; Tomasek *et al.*, 2002; Hinz and Gabbiani, 2003). The actin-containing stress fibers terminate in the fibronexus, which is a specialized transmembrane adhesion complex that links cytoplasmic actin to extracellular fibronectin fibrils through integrins (Singer *et al.*, 1984; Eyden, 1993; Powell *et al.*, 1999). This provides a mechano-transduction system, which transmits the forces generated by the stress fibers to the extracellular matrix (inside-out signaling) (Tomasek *et al.*, 2002). In contrast, extracellular signals may induce an intracellular response (outside-in signaling) (Burridge and Chrzanowska-Wodnicka, 1996; Tomasek *et al.*, 2002). Connections between myofibroblasts are established *via* adherence and gap junctions. The nuclei of myofibroblasts have multiple indentations (Darby *et al.*, 1990; Valentich *et al.*, 1997; Powell *et al.*, 1999). Ordinary fibroblasts lack all of these characteristics (Eyden, 1993; Powell *et al.*, 1999).

Until recently, myofibroblasts were divided into six different subtypes, based on immunohistochemical staining of cytoskeletal proteins (Gabbiani, 1994; Desmouliere and Gabbiani, 1996; Desmouliere *et al.*, 1997). In this classification, one of the myofibroblast subtypes is α-SM actin-negative (Sappino *et al.*, 1990). Currently, it is thought that myofibroblasts should be classified into only two types (Hinz and Gabbiani, 2003). The first type, the proto-myofibroblast, is partly differentiated and contains actin stress fibers but no α-SM actin. This cell type also produces intracellular fibronectin and possesses small fibronexi (Tomasek *et al.*, 2002; Hinz and Gabbiani, 2003). The second type expresses α-SM actin and is considered to be the mature myofibroblast, characterized by an extensive network of stress fibers and large fibronexi (Tomasek *et al.*, 2002; Hinz and Gabbiani, 2003).

(4) FACTORS REGULATING MYOFIBROBLAST DIFFERENTIATION

Factors involved in the differentiation of fibroblasts into proto-myofibroblasts, and subsequently into mature myofibroblasts, are summarized in the Fig. Most factors directly or indirectly influence the expression of α-SM actin, and are therefore involved only in the differentiation of proto-myofibroblasts into mature myofibroblasts.

(4.1) Factors Regulating the Differentiation of Fibroblasts into Proto-myofibroblasts

Mechanical tension generated by migrating fibroblasts promotes the assembly of stress fibers characteristic of the

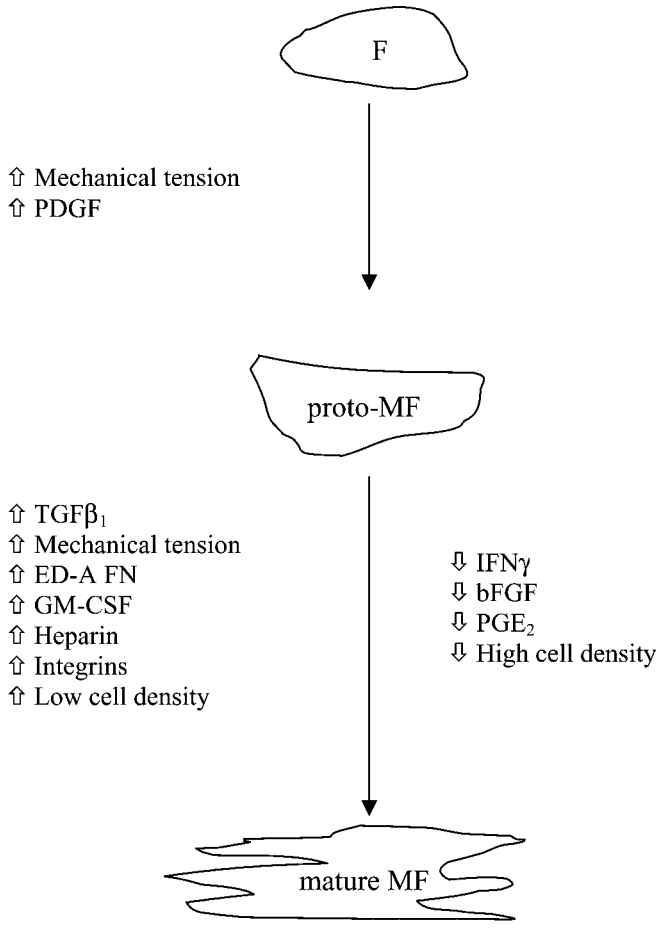

Figure. The myofibroblast differentiation model. ⇧ indicates a stimulation of differentiation and ⇩ an inhibition of differentiation. Abbreviations: F = fibroblast; Proto-MF = proto-myofibroblast; mature MF = mature myofibroblast; PDGF = platelet-derived growth factor; TGF-β = transforming growth factor β; ED-A FN = ED-A (EIIIA) variant of fibronectin; GM-CSF = granulocyte/macrophage-colony-stimulating factor; IFN-γ = interferon-γ; bFGF = basic fibroblast growth factor; PGE_2 = prostaglandin E_2.

proto-myofibroblast (Tomasek *et al.*, 2002; Hinz and Gabbiani, 2003). The increasing numbers of fibroblasts in the wound area secrete new collagen and fibronectin. The orientation of the cells and fibers within this matrix is parallel to the wound surface and along the lines of tension (Hinz *et al.*, 2001). The fibroblasts exert small tractional forces on the newly formed matrix, reinforce cell-matrix contacts, develop intracellular contractile stress fibers, and, hence, become proto-myofibroblasts (Hinz and Gabbiani, 2003). The proto-myofibroblast phenotype is maintained by the continuous interaction between cell-generated tension and the reaction of a substratum that is sufficiently stiff to resist this force (Hinz and Gabbiani, 2003).

The role of platelet-derived growth factor (PDGF) in myofibroblast differentiation (Bostrom *et al.*, 1996; Lindahl *et al.*, 1997) is restricted to the differentiation of fibroblasts into proto-myofibroblasts, since it does not induce the expression of α-SM actin (Tomasek *et al.*, 2002). However, PDGF might be important for the differentiation of keratinocytes into myofibroblasts (Jester *et al.*, 2002). In conclusion, mechanical tension is a major factor promoting the differentiation of fibroblasts into proto-myofibroblasts, and PDGF might also be involved.

(4.2) Factors Promoting the Differentiation of Proto-myofibroblasts into Mature Myofibroblasts

Mechanical tension, TGFβ1, and ED-A FN (a variant of fibronectin) are key players in the differentiation of proto-myofibroblasts into mature myofibroblasts (Tomasek *et al.*, 2002; Gabbiani, 2003; Phan, 2003). TGFβ1 stimulates the expression of ED-A FN and α-SM actin, and it increases the assembly of stress fibers and focal adhesions both *in vitro* and *in vivo* (Borsi *et al.*, 1990; Desmouliere *et al.*, 1993; Ronnov-Jessen and Petersen, 1993; Yokozeki *et al.*, 1997; Vaughan *et al.*, 2000).

ED-A FN is a specific variant of fibronectin that includes the splice segment ED-A, which is expressed only during early embryogenesis (Ffrench-Constant, 1995; Xia and Culp, 1995; Serini *et al.*, 1998) and wound healing (Tomasek *et al.*, 2002). During early embryogenesis, ED-A FN does not induce the differentiation of mature myofibroblasts, because TGFβ1 is absent (Whitby and Ferguson, 1991). Therefore, the role of ED-A FN in myofibroblast differentiation is restricted to post-natal wound healing. Myofibroblasts preferentially bind to ED-A FN *via* the integrins $\alpha_9\beta_1$ and $\alpha_4\beta_1$ (Liao *et al.*, 2002; Muro *et al.*, 2003). In the presence of ED-A FN and TGFβ1, the myofibroblast phenotype is lost when mechanical tension is removed (Hinz *et al.*, 2001). Thus, the differentiation of proto-myofibroblasts into mature myofibroblasts is stimulated by an interplay between TGFβ1 and ED-A FN in the presence of mechanical tension (Serini *et al.*, 1998; Vaughan *et al.*, 2000).

Other factors may also contribute to the differentiation of proto-myofibroblasts into mature myofibroblasts. Granulocyte-macrophage colony-stimulating factor (GM-CSF) seems to induce the synthesis of α-SM actin *in vivo* (Rubbia-Brandt *et al.*, 1991; Feugate *et al.*, 2002), but not when it is added to cultured fibroblasts (Rubbia-Brandt *et al.*, 1991). This suggests that GM-CSF stimulates the clustering of macrophages *in vivo*, which, in turn, express TGFβ1 that stimulates the expression of α-SM actin (Serini and Gabbiani, 1999). Indeed, the appearance of myofibroblasts *in vivo* is often preceded by a cluster-like accumulation of macrophages (Vyalov *et al.*, 1993; Serini and Gabbiani, 1999). Heparin induces α-SM actin *in vitro* (Desmouliere *et al.*, 1992), but, *in vivo*, it requires tumor necrosis factor (TNF)-α (Desmouliere *et al.*, 1992; Schmitt-Graff *et al.*, 1994).

Integrins are also involved in the differentiation of proto-myofibroblasts into mature myofibroblasts (Dugina *et al.*, 2001). The expression of the fibronectin receptor $\alpha_5\beta_1$ increases concurrently with the increase in α-SM actin in differentiating myofibroblasts (Masur *et al.*, 1996, 1999). In fact, large clusters of this integrin are present in the fibronexi of mature myofibroblasts (Dugina *et al.*, 1998, 2001; Masur *et al.*, 1999). The binding of $\alpha_v\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ integrins to vitronectin inhibits the differentiation of mature myofibroblasts (Scaffidi *et al.*, 2001). Function-blocking monoclonal antibodies against these integrins induce the expression of α-SM actin and its organization into stress fibers, which increases the contraction of collagen lattices (Scaffidi *et al.*, 2001). The absence of cell-cell contacts also induces the differentiation into mature myofibroblasts. This is observed if fibroblasts are

plated at a low density (Masur *et al.*, 1996). The absence of cell-cell contacts is thought to cause an increase in the number of TGFβ receptors, resulting in an increase in the expression of α-SM actin by TGFβ (Rizzino *et al.*, 1988).

Cytokines that inhibit myofibroblast differentiation include interferon-γ (IFN-γ) (Hansson *et al.*, 1989), bFGF (Schmitt-Graff *et al.*, 1994; Spyrou and Naylor, 2002), and prostaglandin E_2 (PGE_2) (Kolodsick *et al.*, 2003). Both *in vitro* and *in vivo*, IFN-γ decreases α-SM actin expression (Pittet *et al.*, 1994; Spyrou and Naylor, 2002). Furthermore, IFN-γ decreases collagen lattice contraction by dermal or palatal fibroblasts (Moulin *et al.*, 1998; Yokozeki *et al.*, 1999). Also, bFGF inhibits the differentiation of myofibroblasts *in vitro* (Schmitt-Graff *et al.*, 1994) and *in vivo* (Spyrou and Naylor, 2002; Kanda *et al.*, 2003). Furthermore, bFGF induces apoptosis in myofibroblasts from rat palatal mucosa (Funato *et al.*, 1997). Finally, PGE_2 inhibits the TGFβ1-induced expression of α-SM actin in primary fetal and adult lung fibroblasts (Kolodsick *et al.*, 2003).

In conclusion, mechanical tension is essential for the conversion of fibroblasts into proto-myofibroblasts. The interplay among mechanical tension, TGFβ1, and ED-A FN largely regulates the differentiation of proto-myofibroblasts into mature myofibroblasts (Tomasek *et al.*, 2002).

(5) FACTORS REDUCING WOUND CONTRACTION

The reduction of wound contraction during intra-oral wound healing after cleft palate surgery might be achieved by the inhibition of differentiation of myofibroblasts, either by reducing "stimulatory factors" or by stimulating "inhibitory factors". The stimulation of de-differentiation or apoptosis, or the impairment of myofibroblast function, is another possible approach.

(5.1) Inhibition of Factors that Induce Myofibroblast Differentiation

The absence of TGFβ1 in fetal wounds is related to a reduced differentiation of myofibroblasts (Nodder and Martin, 1997; Moulin *et al.*, 2001; Tomasek *et al.*, 2002). This leads to less wound contraction and scar formation. Elimination of TGFβ1 might therefore prevent wound contraction and scarring and has been studied extensively. Most of these studies report a strong reduction in α-SM actin and therefore a reduction in mature myofibroblasts (Shah *et al.*, 1992; Arora and McCulloch, 1999; Hinz *et al.*, 2003). Neutralizing antibodies against TGFβ1 significantly inhibit collagen lattice contraction by palatal scar fibroblasts (Yokozeki *et al.*, 1997). These antibodies also prevent scar formation during dermal wound healing (Shah *et al.*, 1992). Wounds treated with anti-TGFβ1 showed a lower inflammatory response and less deposition of extracellular matrix in the early stages of wound healing (Shah *et al.*, 1999). However, in the later stages, TGFβ1 induced myofibroblast apoptosis (Funato *et al.*, 1997, 1999). This might result in increased wound contraction and scar tissue formation. Due to this biphasic role of TGFβ1 during wound healing, its elimination may induce new problems, and proper timing of such treatment will be essential.

Another treatment modality might be the blocking of ED-A FN, which is essential for the differentiation of proto-myofibroblasts into mature myofibroblasts (Balza *et al.*, 1988; Serini *et al.*, 1998). An antibody against ED-A FN specifically blocked the TGFβ1-triggered expression of α-SM actin and collagen type I in cultured fibroblasts (Serini *et al.*, 1998). Therefore, the elimination of ED-A FN is a promising approach to improve the outcome of wound healing. Another advantage of ED-A FN is that it is present only in embryos and in wound tissues, which reduces the risk of side-effects (Kornblihtt *et al.*, 1996; Serini and Gabbiani, 1999; De Wever and Mareel, 2002). The relaxation of tension in collagen lattices reduces α-SM actin, ED-A FN, and TGFβ1 levels (Arora *et al.*, 1999; Hinz and Gabbiani, 2003). This indicates that ED-A FN and TGFβ1 are required for the expression of α-SM actin, but tension is also a prerequisite. Since *in vivo* releasing of tension is difficult, the feasibility of this technique for the modulation of wound healing is questionable.

(5.2) Stimulation of Factors that Inhibit Myofibroblast Differentiation

The application of IFN-γ results in a decreased expression of α-SM actin, both *in vitro* and *in vivo* (Pittet *et al.*, 1994; Schmitt-Graff *et al.*, 1994; Moulin *et al.*, 1998; Cornelissen *et al.*, 2000a). Moreover, intralesional injection of IFN-γ decreases the expression of α-SM actin in patients with hypertrophic scars and Dupuytren's disease, which are both characterized by persisting myofibroblasts (Pittet *et al.*, 1994). Also, bFGF inhibits the differentiation of fibroblasts into myofibroblasts, both *in vivo* and *in vitro* (Finesmith *et al.*, 1990; Spyrou and Naylor, 2002). The application of bFGF to a wound results in a tissue architecture more closely resembling that of the normal uninjured situation (Spyrou and Naylor, 2002). PGE_2 is another inhibitory factor for myofibroblast differentiation (Zhu *et al.*, 2001; Kolodsick *et al.*, 2003) and collagen lattice contraction *in vitro* (Zhu *et al.*, 2001). However, the *in vivo* administration of PGE_2 often causes severe side-effects, such as diarrhea, lethargy, and flushing (Paralkar *et al.*, 2003). From the above data, we conclude that bFGF and IFN-γ are the prime candidates for reducing the expression of α-SM actin in myofibroblasts (Moulin *et al.*, 1998).

(5.3) Stimulation of Apoptosis

Myofibroblasts disappear by apoptosis after closure of a wound (Desmouliere, 1995; Desmouliere *et al.*, 1995). A reduction of wound contraction might therefore be achieved by the stimulation of myofibroblast apoptosis (Fesus *et al.*, 1991). In palatal wound healing in the rat, the number of myofibroblasts peaks in the second week after wounding occurs. This coincides with a peak number of apoptotic cells (Van Beurden *et al.*, 2003). The stimulation of myofibroblast apoptosis by TGFβ1 and bFGF might thus reduce the contraction of palatal wounds (Funato *et al.*, 1999). However, as discussed before, TGFβ1 has a biphasic role during palatal wound healing in the rat. Early in wound healing, it stimulates the differentiation of myofibroblasts; after re-epithelialization, it stimulates the apoptosis of myofibroblasts (Funato *et al.*, 1997, 1999). bFGF also stimulates myofibroblast apoptosis (Funato *et al.*, 1997, 1999). Moreover, the application of a mixture of TGFβ1 and bFGF had a synergistic effect (Funato *et al.*, 1997, 1999). Apoptosis can also be stimulated by covering granulation tissue with a vascularized flap (Darby *et al.*, 2002), or by a disruption of cell-matrix interactions (Frisch and Screaton, 2001). Loss of integrin-mediated cell adhesion initiates the response (Frisch and Francis, 1994) and can be

Table. Different Treatment Modalities and Their Clinical Feasibility

Treatment Modality	Feasibility
Neutralize stimulatory factors	
TGF-β_1	+
ED-A FN	++
Mechanical tension	-
Apply inhibiting factors	
IFN-γ	+
bFGF	+
TGF-β + bFGF	+
PGE_2	-
Others	
Apoptosis induction	-
Antibodies	-
RGD-peptides	-

Abbreviations: TGF-β_1 = transforming growth factor β_1; ED-A FN = ED-A (EIIIA) variant of fibronectin; IFN-γ = interferon-γ; bFGF = basic fibroblast growth factor; PGE_2 = prostaglandin E_2.
- not feasible, + promising, ++ most promising.

provoked by RGD-containing peptides (Hadden and Henke, 2000). However, the use of small fibronectin peptides deserves caution, since all integrin-mediated cell-matrix interactions may be disrupted.

(5.4) Impairment of Myofibroblast Function

Impairment of myofibroblast function might be achieved by the modification of integrins on the cell surface. Integrins mediate the mutual contacts among myofibroblasts, and the contacts between myofibroblasts and the surrounding matrix (van der Flier and Sonnenberg, 2001). The integrins $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$ (Horwitz, 1997), and $\alpha_8\beta_1$ (Levine *et al.*, 2000) thus allow for the transmission of contractile forces through the wound (Racine-Samson *et al.*, 1997). The binding of antibodies to these integrins might reduce wound contraction by preventing matrix contraction, or by preventing myofibroblast differentiation (Arora *et al.*, 1999). However, the same integrins on other cell types will also be blocked. Local application of the antibodies and proper timing of the treatment might reduce some of these side-effects.

Integrin function can also be modified with peptides containing the Arg-Gly-Asp (RGD) sequence. This sequence is a major recognition site for integrins (Arnaout *et al.*, 2002). Since the integrin-mediated cell attachment also regulates fibroblast differentiation, simple RGD peptides might be used to prevent the appearance of myofibroblasts. A drawback of RGD peptides is that they may also affect other cell types, which causes unwanted side-effects. However, the performance of RGD peptides can be enhanced by stereochemical changes, which influence their efficiency and specificity (Williams, 1992). In conclusion, RGD peptides may reduce the attachment of myofibroblasts to the matrix, and thereby diminish wound contraction. This is supported by the inhibition of retinal pigment epithelial cell-mediated collagen lattice contraction by a disintegrin (Yang *et al.*, 1997). However, the use of natural or artificial peptides containing the RGD sequence may cause severe side-effects.

(5.5) Other Techniques

Several other techniques with a possible beneficial effect on wound healing have been studied. They influence different aspects of the wound-healing process and include low-level laser therapy (In de Braekt *et al.*, 1991), biodegradable membranes (In de Braekt *et al.*, 1991, 1992), matrix substitutes (Fujioka and Fujii, 1997), and modified surgical techniques (Leenstra *et al.*, 1995; Noguchi *et al.*, 2003). The covering of the wound with a vascularized flap or a tissue-engineered product may also reduce wound contraction (Darby *et al.*, 2002; Von den Hoff *et al.*, 2005). Fetal surgery might be an option, since fetal wounds have been shown to heal without scarring. However, it is questionable whether this technique should be used in the treatment of non-life-threatening congenital anomalies (Weinzweig *et al.*, 1999), of which the pre-natal cleft diagnosis is rather inaccurate. Additionally, the ethical problems related to fetal repair of clefts have not yet been solved (Molsted, 1999).

(6) CONCLUDING REMARKS

The surgical closure of a cleft palate is considered to impair maxillary growth and dento-alveolar development (Ross, 1987; Wijdeveld *et al.*, 1991). Both wound contraction and scarring contribute greatly to these undesirable effects of surgery (Wijdeveld *et al.*, 1987a, 1991). This review focuses on the role of myofibroblasts during the oral wound-healing process, and, more specifically, on their role in wound contraction. The reduction of wound contraction might improve the outcome of the oral wound-healing process. Recently, it has been described that fibroblasts initially differentiate into proto-myofibroblasts and subsequently into mature myofibroblasts, both of which contribute to wound contraction (Tomasek *et al.*, 2002; Gabbiani, 2003). Several factors that regulate these differentiation pathways are now known. Some of them might be promising as targets for therapeutical intervention and are described in this review. The Table summarizes these possible treatment modalities. Some of them seem to be feasible for therapeutic intervention, while others have too many disadvantages. The application of TGFβ inhibitory factors is often described as a promising tool to reduce the differentiation of fibroblasts into mature myofibroblasts. However, due to its bivalent role in wound healing, the application of this cytokine is not without risk. Among the more promising growth factors are IFN-γ and bFGF, because both factors have been shown to inhibit the differentiation of proto-myofibroblasts into mature myofibroblasts, both *in vivo* and *in vitro.* Blocking of ED-A FN seems to be most promising, because the risk of side-effects will be negligible, since it is expressed exclusively during wound healing. Future studies should focus on the topical application of these promising substances, or on their incorporation into suitable carrier materials. Recently, it was shown that cells originating from the bone marrow also contribute to wound healing and possibly also to fibrosis (Fathke *et al.*, 2004; Ishii *et al.*, 2005). This might offer new prospects for therapy.

Most of the research on myofibroblasts has been performed on skin, although some research on oral mucosa has also been published. The differences and similarities between wound healing in the dermis and that in the oral cavity are discussed in this review. The most remarkable differences are that wounds in the oral mucosa heal faster and with less scarring than do

dermal wounds. In spite of this, the specific features of the palatal mucoperiosteum give rise to extensive wound contraction and scar tissue formation. This knowledge should be borne in mind for the development of therapeutic interventions to improve the outcome of cleft palate surgery.

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