REVIEW ARTICLE

Tissue engineering strategies combining molecular targets against inflammation and fibrosis, and umbilical cord blood stem cells to improve hampered muscle and skin regeneration following cleft repair

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Abstract
Cleft lip with or without cleft palate is a congenital deformity that occurs in about 1 of 700 newborns, affecting the dentition, bone, skin, muscles and mucosa in the orofacial region. A cleft can give rise to problems with maxillofacial growth, dental development, speech, and eating, and can also cause hearing impairment. Surgical repair of the lip may lead to impaired regeneration of muscle and skin, fibrosis, and scar formation. This may result in hampered facial growth and dental development affecting oral function and lip and nose esthetics. Therefore, secondary surgery to correct the scar is often indicated. We will discuss the molecular and cellular pathways involved in facial and lip myogenesis, muscle anatomy in the normal and cleft lip, and complications following surgery. The aim of this review is to outline a novel molecular and cellular strategy to improve musculature and skin regeneration and to reduce scar formation following cleft repair. Orofacial
Clefting can be diagnosed in the fetus through prenatal ultrasound screening and allows planning for the harvesting of umbilical cord blood stem cells upon birth. Tissue engineering techniques using these cord blood stem cells and molecular targeting of inflammation and fibrosis during surgery may promote tissue regeneration. We expect that this novel strategy improves both muscle and skin regeneration, resulting in better function and esthetics after cleft repair.

**KEYWORDS**
cleft lip and palate, oral surgery, scarring, tissue engineering, umbilical cord blood stem cells

### 1 | INTRODUCTION

Cleft lip with (CLP) or without cleft palate (CL) occurs in about 1 to 700 newborns with ethnic and geographical variation and is one of the most common facial congenital anomalies.\(^1\)\(^-\)\(^3\) A CL is present in about 0.6 per 1000 live births.\(^4\) CL(P) is either uni- or bilateral (Figure 1) and can occur isolated or as part of a syndrome.\(^5\) Since only 30%...
of children born with CLP have a genetic syndrome, a combination of genetic and environmental factors is thought to play a role in the etiology of orofacial clefting.6,7 These environmental risk factors include smoking,8 alcohol consumption,9 phenytoin exposure,10 diabetes,11 and maternal12 and paternal age.13 Other factors such as folic acid supplementation, zinc, and daily multivitamin intake, can reduce the risk of CL(P).14

There is a large variation in the severity of the cleft lip, ranging from mild subepithelial to complete bilateral clefting (Figure 1). The more severe the cleft lip, the more the shape and size of the alveolar process are affected. The cleft in the alveolar process can range from a small dimple in the arch in combination with a minor cleft of the lip to a total cleft of the alveolar ridge and anterior displacement of the premaxilla (Figure 1).14 Cleft lip and primary or secondary palate clefts differ in embryonic origin and underlying fusion or differentiation defects. Fusion defects of the primary palate lead to complete clefting of the lip either or not combined with a complete or incomplete clefting of the alveolus. Differentiation defects of the primary palate give rise to incomplete, submucous or hypoplastic cleft lip and/or alveolus. Fusion defects of the secondary palate lead to complete or incomplete hard-palate clefts that may be combined with a cleft in the soft palate and/or uvula. Differentiation defects of the secondary palate give rise to a combination of submucous, hypoplastic hard and soft palate defects.15–17

If the cleft is not surgically corrected, patients are more prone to hearing problems due to otitis media with effusion,18 and have difficulties with speech, feeding, and abnormal dental development due to loss of lip pressure. In addition, they may encounter social isolation. Clefts can cause serious psychological problems including low self-esteem and acceptance by peers.19,20

Scarring following surgical correction of the cleft lip can have a major effect on subsequent anterior-posterior and vertical growth of the maxilla, as well as on the position of the maxillary incisors.21 Excessive pressure of the repaired upper lip leads to palatal inclination of the upper front teeth and an increased overbite but decreased overjet.22 When the patient grows older a reversed overjet often develops due to growth restriction of the maxilla, especially in CLP cases. Several factors contribute to suboptimal lip muscle repair including the skills and experience of the surgeon,23 type and extension of the cleft,24 genetic factors,25 muscle fiber type distribution, and impaired muscle regeneration.26,27 In this review, we therefore present a novel tissue engineering strategy that aims to prevent scarring and subsequent functional problems following cleft surgery by promoting muscle and skin regeneration.

2 | FACIAL AND LIP MYOGENESIS

Orofacial development involves the migration, proliferation, differentiation, and apoptosis of mesenchymal and epithelial cells.28 From the 4th week of gestation cranial neural crest cells (CNCCs) migrate from the dorsal part of the neural tube to form the human face. Next, these CNCCs differentiate into the mesenchymal cells that form structures such as cartilage and bone. The cranial paraxial mesoderm (CPM) provides the precursors for the cranial muscles. Both CNCCs and CPM form the templates for the adult craniofacial structures in the branchial arches.29 In the 5th week of gestation, the CNCCs form the frontonasal prominence, the bilateral maxillary prominences and the bilateral mandibular prominences. In the 6th and 7th week the medial nasal prominences grow and fuse with each other to form an intermaxillary segment. This intermaxillary segment will later fuse with the maxillary prominences and form the upper lip. Any disruption in these well-orchestrated processes can lead to a cleft in lip, alveolus and/or palate.28,30–32

The muscles of the upper lip are all derived from the mesoderm of the 2nd pharyngeal arch and partly determine facial expression (Figure 2).33,34 Mesenchymal condensations that form the individual upper lip muscles emerge sequentially, starting from the 6th week of gestation in human.35 During the development of the facial muscles, these mesenchymal condensations differentiate and move to their definitive location where they mature.34

In summary, the main muscles of the upper lip originate from mesenchymal condensations in the pharyngeal arches.
3 | MUSCLE ANATOMY IN THE NORMAL AND CLEFT LIP

The upper lip muscles comprise the orbicularis oris and seven pairs of other facial and lip muscles. The facial muscles are divided into a superficial (extrinsic) layer and a deep (intrinsic) layer, which work either synergistically or antagonistically in the coordination of lip movements. The orbicularis oris muscle is the primary muscle of the lip and therefore the most important muscle in cleft lip surgery. Facial expressions and lip movements during speaking are supported by the superficial part of the orbicularis oris, while mastication is mediated by the deep layer. The superficial muscle fibers end up in the philtrum ridge on one side, whereas the deep layers cross the midline to insert on the opposite side. The philtrum dimple originates from a reduced attachment of muscles to the surface.

The orbicularis oris muscle contains both slow and fast fibers. The slow fibers are more resistant against fatigue and have a low activation threshold, whereas the fast fibers are more prone to fatigue and have a high activation threshold. Both the superficial and deep orbicularis oris muscle are predominantly composed of fast fibers, but there is a slight difference in fiber type composition between the superficial (98% fast) and deep muscle layers pars peripheralis (95% fast) and pars marginalis (91% fast). This indicates that these muscles should be reconstructed separately during surgery.

In a cleft lip, all facial muscle fibers lack their insertions in the midline and therefore have an abnormal orientation on one or both sides (uni/bilateral cleft, respectively) (Figure 2). The orbicularis oris normally functions as a sphincter but, in a cleft lip, it pulls the two portions of the lip laterally leading to an imbalance between lip and tongue pressure. When a cleft lip is not surgically corrected at a young age, the anterior teeth will often become malpositioned.

The superficial fibers of the orbicularis oris muscle are oriented parallel to the cleft, medial towards the columella and lateral to the alar base. The fibers at the medial side are deficient. These superficial fibers are more disorganized with increasing severity of the cleft. By contrast, the orientation of the deep layer of the orbicularis oris remains unchanged. This muscle runs perpendicular to the cleft and ends at the edges of the cleft (Figure 2).
In cleft lip, an increased amount of fast muscle fibers is present, which has also been demonstrated in the musculus levator veli palatini of the cleft soft palate. In vitro studies have shown that satellite cells (SCs) from fast muscle fibers proliferate less than those from slow muscle fibers. This, together with the reduced capillary supply and disorganization of the muscle fibers may compromise muscle regeneration in a cleft lip after surgical repair.

Summarizing, in CL(P) the orientation, insertion, and composition of the muscles of the lip are abnormal, which hampers muscle regeneration after surgery, affecting the oral function and dental development.

4 | CLEFT SURGERY CAN RESULT IN FIBROSIS AND SCAR FORMATION

The two main techniques in unilateral cleft lip surgery are the triangular flap technique (Tennison-Randall) and the rotation advancement technique (Millard), which have similar outcomes. Common bilateral cleft lip techniques include the Millard technique, the Manchester technique and the Tennisson technique. A cleft lip is often accompanied by a cleft palate and cleft alveolus, and is treated at different time points after birth depending on the treatment protocol.

Surgery of the lip usually takes place in the first year after birth, often in conjunction with the repair of the nose and the soft palate. The hard palate is closed as late as possible, taking into account the speech development of the patient and the potential negative effects on facial growth. There is a large variation in the timing of surgical treatments between CLP surgical centers. However, the outcome is often suboptimal, depending on the severity of the original defect and the displacement of the cleft maxilla. Dehiscence of the lip or the palate shortly after surgery or scar formation with subsequent hampered growth of the maxilla are found. Revision surgery is therefore required in 33% of the CLP patients compared to 12% of the patients with an intact secondary palate.

Normally, wound healing of skin and muscle is characterized by four successive and overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Disruption of blood vessels as a result of cleft surgery causes bleeding. Next, a blood clot containing fibrin will form by platelet aggregation. During hemostasis, platelets release cytokines and growth factors that attract inflammatory cells like granulocytes and macrophages. These inflammatory cells remove apoptotic cells, debris, and invading bacteria. Released cytokines and growth factors then stimulate keratinocyte and fibroblast proliferation, and angiogenesis to promote tissue regeneration. This proliferative phase consists of epithelial proliferation, the formation of granulation tissue and epithelialization. These processes are regulated by fibroblasts and endothelial cells. Fibroblasts produce collagen, glycosaminoglycans and proteoglycans, the major components of the extracellular matrix (ECM). Mechanical strain, transforming growth factor beta 1 (TGF-β1) and the ECM molecule ED-A fibronectin induce the differentiation of fibroblasts into myofibroblasts. Myofibroblasts contract the granulation tissue and deposit large amounts of ECM molecules. Subsequently, matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPS) control the remodeling process. During remodeling cross-linking and reorientation of collagen fibers occurs, while the number of capillaries regresses returning the vascular density to normal. The immature type III collagen is modified to mature type I collagen. At the end of this phase, the myofibroblasts die by apoptosis leaving a rather acellular scar. However, multiple molecular and mechanical factors can affect wound healing after CL(P) surgery and promote fibrosis and scar formation resulting in major functional and aesthetic problems.

In normal skin, ECM deposition and degradation are balanced. An accumulation of collagen type I and III fibers and other ECM proteins after injury results in a disorganized fiber structure and hypertrophic scar formation. Wound tension or mechanical stress is another causative factor for scar formation. The skin of the human face is maximally extensible perpendicular to relaxed skin tension lines, implying that tension is minimized when surgical incisions are created along these lines.

Prolonged inflammation and oxidative stress blocks the remodeling phase and promotes excessive fibrosis and scarring. Therefore, stringent control of oxidative and inflammatory factors is important for the outcome of wound repair in skin and muscle. Several factors including the cytokines interleukin-1 beta (IL-1 β), interleukin-6...
(IL-6), tumor necrosis factor alpha (TNF-α), TGF-β1, monocyte chemoattractant protein-1 (MCP1) and heme can induce fibrosis and scarring. These factors promote inflammatory cell adhesion, migration/proliferation of leukocytes and fibroblasts, and dysregulation of ECM remodeling. Thus, to prevent excessive scarring, we need to limit the inflammatory and fibrotic processes and promote regeneration.

5 | STEM CELLS REPAIR WOUNDS AND FACILITATE SKIN AND MUSCLE REGENERATION

Stem cells facilitate maintenance and repair processes in skin and muscle by replenishing lost tissue and by creating a regenerative microenvironment. Stem cells have a prolonged self-renewal capacity and are able to differentiate into various cell types, making them ideal for use in regenerative medicine. Stem cells are required at the site of injury to allow the regeneration of dermal, epidermal and muscle tissue. Moreover, they promote scarless wound healing by generating a regenerative microenvironment via the secretion of protective factors that can inhibit myofibroblasts in a paracrine fashion.

Following skin wounding, epidermal stem cells in the basal layer are activated, proliferate and migrate to the site of injury where they contribute to regeneration. Muscle repair following injury is facilitated by SCs. Satellite cells are muscle stem cells originating from the CPM that are responsible for postnatal muscle growth, maintenance, and repair. They express the paired box transcription factor 7 (Pax7) and are located between the sarcolemma and basal lamina surrounding a single muscle fiber. After the injury, SCs become activated and migrate to the site of injury, proliferate, and differentiate into myoD-positive myoblasts that fuse to form new multinucleated myosin heavy chain (MyHC)-positive myofibers or repair damaged myofibers (Figure 3). A small portion of these SCs stay quiescent to allow future regeneration cycles. Signaling molecules like insulin-like growth factor 1 and 2 (IGF-1, IGF-2), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) derived from recruited macrophages, injured myofibers and disrupted ECM regulate this process. HGF induces the activation of quiescent SCs. IGF-1 and 2 to enhance myogenic proliferation and differentiation, and promote cell survival. PDGF regulates the proliferation and differentiation of myoblasts and supports angiogenesis. FGF is upregulated during muscle regeneration, but its exact role is unclear.

Muscle-specific transcription factors like Pax7, myogenic factor 5, MyoD, myogenin, and finally structural proteins like MyHC are sequentially expressed (Figure 3). Most of the studies on muscle regeneration have been performed on skeletal muscles of limb and trunk. During embryonic development, the limb and trunk muscles are derived from the somites whereas lip muscles are derived from the CPM in the branchial arches. Head muscles also contain less SCs than limb muscles and injuries regenerate slower. In addition, Pax7 and myogenic regulatory factor 4 levels in limb muscles are lower than in

FIGURE 3  Immunofluorescence staining of muscle fiber formation from masseter satellite cells (SCs): Left: after 3 days of culture, DAPI (blue) stains nuclei, Pax 7 (red), Middle: after 7 days of culture, DAPI (blue), MyHC (red), Right: after 10 days of culture DAPI (blue), MyHC (red). Magnification ×400. DAPI, 4′,6-diamidino-2-phenylindole; MyHC, myosin heavy chain; Pax 7, paired box transcription factor 7 [Color figure can be viewed at wileyonlinelibrary.com]
head muscles. SCs from the masseter muscle proliferate more and differentiate later than those from limb muscles. SCs from different branchiomeric muscles, however, behave in a similar way. The lower regenerative capacity of branchiomeric muscles compared to limb muscle may impair muscle healing after cleft surgery. The reduced blood supply and aberrant muscle orientation in a cleft lip may further impair the regeneration process. Thus, stem cells could provide a solution for skin repair, but also for muscle repair.

**6 | REGENERATIVE MEDICINE TO ATTENUATE SCARRING AND MUSCLE FIBROSIS**

To overcome the functional and esthetic problems related to scarring after cleft lip surgery, we present a novel tissue engineering strategy. This involves a combination of umbilical cord blood stem cells with regenerative capacity together with anti-inflammatory and antifibrotic molecules.

In comparison to adults, fetuses display less scarring following wound healing. Fetal wounds differ in several ways from adult wounds, including a reduced inflammatory response. They also have higher expression levels of MMPs compared to their inhibitors, the TIMPs. Further, PDGF disappears more rapidly from fetal wounds, that also show lower FGF levels and increased TGF-β3 levels. In addition, the level of TGF-β1/2 is lower, the biomechanical strain is lower and the ECM is enriched in hyaluronic acid and type III collagen.

Tissue regeneration is often hampered by the occurrence of fibrosis. TGF-β1 is a pro-inflammatory factor that recruits macrophages and other inflammatory cells to the wound site, thus contributing to fibrosis and excessive scarring. In muscles, TGF-β1 stimulates the synthesis of collagen and other ECM components and promotes myofibroblast formation and fibrosis. Myostatin, also a member of the TGF-β family inhibits muscle regeneration by reducing the proliferation of stem cells and myoblasts. The inhibition of myostatin and TGF-β may enhance muscle regeneration. The proteoglycan decorin has a stimulatory effect on autophagy and inflammation, and an inhibitory effect on angiogenesis by inhibiting both TGF-β1 and myostatin. This protein can be used to prevent fibrosis and enhance muscle regeneration. Decorin also upregulates myogenic genes and promotes skeletal muscle regeneration after injury. Losartan, an angiotensin II receptor antagonist, neutralizes the effect of TGF-β1, reduces fibrosis and has already been used clinically in therapy for myocardial fibrosis.

Oxidative stress and inflammation can be counteracted by the induction of heme oxygenase (HO) by metalloporphyrins. The enzyme HO degrades free heme generating carbon monoxide, ferrous iron/ferritin, and biliverdin/bilirubin, which can be used to reduce inflammation. HO-1 deficient humans and mice demonstrate chronic inflammatory stress. HO-1 has pro-angiogenic effects via regulating vascular endothelial growth factor (VEGF) synthesis. HO-2 deficient mice have elevated levels of pro-inflammatory chemokines including keratinocyte chemoattractant, macrophage inflammatory protein 2 (MIP-2), and MCP-1. These mice show delayed wound healing and an exaggerated inflammatory response after corneal epithelial wounding. Alternatively, the systemic administration of anti-inflammatory therapeutics during surgery may attenuate scarring. These include neuropeptide α-melanocyte stimulating hormone, FGF9, WNT 3A, interleukin 10 (IL-10), interleukin 6 (IL-6), anti-VEGF antibody, TGFβ3 and CXC chemokine receptor type 4 antagonists. IL-10 downregulates pro-inflammatory cytokines IL-6 and IL 8 and may thus attenuate scar formation.

TGF-β1 is the main target in antifibrotic therapies that include the administration of FGF basic, insulin IGF-1, losartan, suramin, decorin, relaxin, interferon-γ, and angiotensin II receptor blockers. Antagonists of the IL-1 receptor (anakinra; Food and Drug Administration (FDA) approved) reduces TGF-β1 expression and could be used for the reduction of scar formation. Over the years, various drugs have been developed that target profibrotic signaling pathways such as TGF-β and PDGF, to reduce the progression of fibrosis. Still, hardly any drugs are available for the treatment of organ...
Current treatments for idiopathic pulmonary fibrosis, namely pirfenidone and nintedanib. Pirfenidone, that is, pyridine(5-methyl-1-phenyl-2-(1H)-pyridone), is an oral agent that can elicit anti-inflammatory, antioxidative, and antiproliferative effects. Even though, the exact mechanism of action is still not fully understood, the antifibrotic effect of pirfenidone has been demonstrated in several organs, for example lung, liver, and kidney. In human liver cells, it has been shown that it attenuates TGF-β-induced mRNA expression of α-SMA and type I collagen. Also in human precision-cut liver slices a reduction in the gene expression of type I collagen was observed following treatment with pirfenidone.

Nintedanib, formerly known as BIBF 1120, is an intracellular triple angiokinase inhibitor targeting the VEGF, FGF, and PDGF receptors. Nintedanib competitively binds to the adenosine triphosphate binding pocket of these receptors inhibiting their activation and thereby inhibiting the fibrotic process. In addition, nintedanib has also been demonstrated to inhibit members of the proto-oncogene tyrosine-protein kinase (Src)-family (Src, Lyn, and Lck), which are activated in various types of cancer and belong to the non-receptor tyrosine kinases.

Galunisertib, LY2157299, is an oral small molecule inhibitor of the TGF-β receptor I kinase that specifically inhibits SMAD2 phosphorylation. Using human precision-cut liver slices, it has been demonstrated that galunisertib attenuates SMAD2 phosphorylation in healthy and cirrhotic liver tissue and reduces the expression of several collagen subtypes (collagen types I, III, IV, V and VI). In addition, it reduces the expression of numerous genes associated with collagen maturation and homeostasis. Interestingly, there is also evidence suggesting that galunisertib can inhibit TGF-β signaling in human oral keratinocytes.

Overall, there are three distinct routes to target profibrotic signaling: (a) inhibiting transcription or translation of profibrotic factors, (b) blocking interleukin receptor binding and activation, and (c) interfering with down-stream signaling events. Since the fibrotic process is highly similar in all organs, therapeutic targets identified in, for instance, the liver might also be used to reduce scar formation following cleft surgery. The three drugs described above have proven antifibrotic efficacy and are therefore ideal candidates for further study in the context of cleft repair.

7 | UMBILICAL CORD: FROM WASTE MATERIAL TO SOURCE OF THERAPEUTIC STEM CELLS

Adult stem cells like mesenchymal stem/stromal cells (MSC) can be found throughout the body. Mesenchymal stem cells are currently widely used for regenerative medicine purposes. They may stimulate the regeneration of the injured skin and muscle following surgical lip closure by replenishing the required cells and by providing a microenvironment that attenuates scarring and fibrosis. MSCs can differentiate into several types of cells including endothelial cells, myocytes, keratinocytes, and fibroblasts. Moreover, stem cells produce several anti-inflammatory and antifibrotic mediators, and secrete factors that create a regenerative microenvironment. Cord blood stem cells embedded in a scaffold secrete for example MMP-9, mediating collagen degradation in uterine scars, which improves the regeneration of the endometrium, myometrium and blood vessels. Administered cord blood stem cells also promote the recruitment of endogenous stem cells. The isolation of MSCs from bone marrow or adipose tissue from the baby is rather invasive and can cause complications such as infection, bleeding, and chronic pain. Moreover, it is expensive to harvest them and to expand them by controlled cell culture. Therefore, there is a need for alternative sources of MSCs. Human umbilical cord blood cells are obtained by a simple, safe, and painless procedure upon birth. Mesenchymal stem cells can be easily isolated from cord blood and preserved for later. Since prenatal ultrasound screening allows early detection of a cleft in the 11th to 13th week of gestation, the preservation of umbilical cord (blood) upon birth can be planned in an early stage and should be considered as a new strategy to optimize cleft surgery (Figure 4). Cord blood can be collected from the umbilical vein or from the placenta. Only the mononuclear fraction
which contains the stem cells is needed for preservation. In addition, stem cells can be harvested simultaneously from the umbilical cord tissue itself. The umbilical cord mesenchymal stem cells (UCMSC) can be taken from the amniotic membrane, the cord lining, Wharton’s jelly, and the perivascular region of the umbilical cord. The umbilical cord blood and tissue contain a heterogeneous mixture of stem and progenitor cells at different stages of differentiation. It is an attractive source of stem cells because it is considered biowaste accompanying the delivery of a baby. We propose here that these UCMSCs could be used during CL(P) surgeries to facilitate skin and muscle regeneration (Figure 4).

The successful use of human umbilical cord blood and tissue mesenchymal stem cells (hUCMSC) has been shown for other fields in a variety of in vitro, animal, and human studies. It has been demonstrated that umbilical cord blood cells can differentiate into epithelial cells, osteoblasts and adipocytes and also have a myogenic potential. In a study on Duchenne Muscular Dystrophy, myoblasts from Duchenne and Becker muscular dystrophy patients were isolated from the biceps, cocultured with hUCMSCs obtained from healthy babies and analyzed by immunofluorescence microscopy. After 15 days of culturing, dystrophin-positive myofibers were present demonstrating the differentiation of hUCMSCs into muscle cells in vitro. In another in vitro study, hUCMSCs—derived mononuclear cells gave rise to fibroblast-like cells expressing mesenchymal antigens. When these cells were cultured under promyogenic conditions they expressed myogenic markers like MyoD, MyoG, and MyHC. This indicates that hUCMSCs can differentiate into myogenic stem cells in vitro, which could possibly facilitate regeneration of the orbicularis oris muscle after cleft lip surgery.

In vivo, the healing of deep burn wounds in adult Wistar rats was improved with intravenously injected cord blood stem cells compared to controls. The hUCMSCs migrated into the wound and decreased the quantity of inflammatory cells, while neovascularization was increased compared to the control groups. A biomimetic scaffold with hUCMSCs and fibrin showed an improved full-thickness skin wound repair without scar formation in rats compared to controls without scaffold or with a scaffold without hUCMSC. Similarly, hUCMSCs in

**FIGURE 4** Following detection of a cleft lip and/or palate (CL/P) using prenatal ultrasound screening, cord blood stem cells are isolated upon birth. These stem cells express antifibrotic factors and anti-inflammatory factors and can be used in tissue engineering strategies in combination with factors targeting inflammation and fibrosis (e.g., anakinra, pirfenidone and nintedanib) within a scaffold during cleft surgery to promote skin and muscle regeneration and function and to inhibit scar formation [Color figure can be viewed at wileyonlinelibrary.com]
combination with a collagen-fibrin double layer membrane and a single layer collagen membrane resulted in accelerated wound repair in mice when compared to mice treated without hUCMSC.\textsuperscript{149}

The application of hUCMSCs within a small intestinal submucosa-derived ECM scaffold to a full thickness excisional wound in mice also enhanced wound repair and angiogenesis at the wound site. An increase in angiogenic growth factors like HGF, VEGF, and angiopoietin was observed.\textsuperscript{150} Wharton's jelly mesenchymal stem cells also upregulated genes involved in re-epithelialization, neovascularization, fibroblast proliferation, and migration in an excisional full thickness murine wound model.\textsuperscript{151} All these in vivo animal studies support the use of umbilical cord stem cells as a novel wound repair strategy.

In patients with Crohn's Disease, the intravenous infusion of allogeneic hUCMSCs improved disease conditions with only mild side effects like fever and respiratory tract infection.\textsuperscript{152} In atopic dermatitis, a chronic and relapsing skin disease involving pruritus, xerosis, and eczematous lesions, infusion of hUCMSCs was effective without side effects.\textsuperscript{150} Moreover, also allogeneic hUCMSCs have been successfully used in multiple sclerosis patients improving the patients' symptoms without serious adverse effects.\textsuperscript{153}

Besides effectiveness, the question should also be answered whether it is safe to use hUCMSCs. In children with cerebral palsy (motor disorder in childhood), autologous hUCMSCs improved whole brain connectivity and motor function.\textsuperscript{154} Similar studies found that infusion of autologous cord blood cells in children with autism spectrum disorder improved behavior and was safe.\textsuperscript{155,156} A 6-year follow-up study also demonstrated the long-term safety profile of hUCMSCs infusion for drug-resistant systemic lupus erythematosus patients. The treatment reduced the disease activity significantly.\textsuperscript{157} This confirms that the use of hUCMSCs is thus safe and no tumor-related effects occur.\textsuperscript{157} A study with allogeneic hUCMSCs in an osteoarthritic patient showed regeneration of cartilage without adverse effects up to 7 years of follow up.\textsuperscript{158} Allogenic cord blood stem cell transplantation was also successfully used in babies with severe combined immunodeficiency syndrome and led to a reconstitution of hematopoietic cells.\textsuperscript{159,160} No long-term graft vs host disease was observed.

Summarizing, the umbilical cord tissue and umbilical cord blood is an easily accessible source of MSC for the regeneration of skin and muscle tissue. These stem cells can differentiate into the required cell types and secrete regenerative factors.\textsuperscript{143,145,146} hUCMSCs are save for use in children and adults.\textsuperscript{154–157} while no immunogenic rejection was observed after allogeneic transplantation.\textsuperscript{152,153} The low immunogenicity of hUCMSCs would also allow the use of hUCMSCs from a central cord blood stem cell bank when autologous stem cells are not available.

We therefore postulate that cord blood stem cells can be used as a novel adjuvant strategy in cleft surgery to prevent fibrosis and scarring to improve the outcome of the surgery. This will eventually lead to a better quality of life for patients with CL(P).

8 CLINICAL CHALLENGES FOR SCAFFOLD-BASED CORD BLOOD STEM CELLS WITH ANTI-FIBROTIC/ANTI-INFLAMMATORY FACTORS

Direct injection of cord blood stem cells into a wound site is not always effective.\textsuperscript{134} In muscle tissue, isolated stem cells can be applied to the site of injury to improve regeneration.\textsuperscript{161} However, the success rate after isolation and injection of stem cells has been low. Stem cells seem to lose their migratory and regenerative potential and often die.\textsuperscript{162}

Thus, stem cell therapy is complicated by the limited number of cells surviving after administration at the wound site. This is due to the harsh conditions within the wound microenvironment characterized by a wealth of pro-oxidative and pro-inflammatory mediators, and only limited blood flow.\textsuperscript{163–165} Stem cells inducing anti-apoptotic, anti-inflammatory, and anti-oxidative genes can better withstand these insults and have a better chance of surviving.\textsuperscript{163}

Cytoprotective genes include enzyme systems such as HO-1, glutathione S-transferase, dismutases, catalases, and peroxidases. HO-1 induction improves stem cell survival and improves the outcome of stem cell
In contrast, abrogation of HO-activity decreases the efficacy of stem cell therapy. Preconditioning strategies in which cytoprotective pathways (e.g. via nrf2 activation) are activated in cord blood stem cells could, therefore, promote their survival and achieve a better outcome of cleft surgery. Alternatively, the administration of antioxidants (vitamins A, C, and E, glutathione/NAC, mannose-specific lectins, and bilirubin) could protect stem cells from dying.

Anti-inflammatory and antifibrotic factors and stem cells can be applied in a scaffold. Scaffolds are used as an artificial ECM, which supports cell attachment, proliferation, and differentiation of cells. The scaffold guides the newly formed tissue and allows ingrowth of blood vessels and nerves crucial for cell survival. Different types of scaffolds are available prepared from a wide range of biomaterials. Skin and muscle scaffolds can be of natural origins such as fibrin, alginate or collagen, or can be synthetic such as polypropylene, polyesters, polyurethanes, and polysiloxanepeptide hydrogel. They can be produced with a variety of methods, such as 3D printing and electro-spinning. Synthetic biomaterials can be degradable or nondegradable. The advantages of synthetic biomaterials are their low immunogenicity and reproducible quality, while they can be custom made with the required mechanical properties and shape. Scaffolds can be seeded with cord blood stem cells together with the anti-inflammatory and anti-fibrotic factors to create a regenerative microenvironment that facilitates tissue regeneration and prevents scar formation. Tissue engineering/regenerative medicine scaffolds require the appropriate physical and cellular signals to promote tissue regeneration. The sum of the biochemical signals and biophysical cues from the microenvironment dictate the fate of stem cells. The sum of pro- and anti-inflammatory and fibrotic factors, the shape of scaffolds, stiffness of the matrix, nanotopography and the presence of biofunctional groups as RGD-sequences discriminates between scarring and regeneration of tissue. Mechanical cues may be applied in tissue engineering by adjusting the type, stiffness and architecture of the scaffolds facilitating the differentiation of cord blood stem cells into either skin or muscle tissue.

9 | CONCLUSIONS

The functional and esthetic outcome of cleft palate repair is often hampered by fibrosis and scarring. In this review, we discuss novel tissue engineering strategies to promote muscle and skin regeneration, while preventing scarring. Umbilical cord blood stem cells are a promising, safe, and non-invasive source of stem cells that can be combined in a scaffold together with anti-inflammatory and anti-fibrotic molecules. Overexpression of cytoprotective molecules in these stem cells by preconditioning before applying them to the wound area may not only increase stem cell survival but also further contribute to a regenerating microenvironment. We expect that this novel strategy improves both muscle and skin regeneration after cleft repair resulting in a better functional and esthetic outcome.

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