Orofacial Muscles: Embryonic Development and Regeneration after Injury

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Abstract

Orofacial congenital defects such as cleft lip and/or palate are associated with impaired muscle regeneration and fibrosis after surgery. Also, other orofacial reconstructions or trauma may end up in defective muscle regeneration and fibrosis. The aim of this review is to discuss current knowledge on the development and regeneration of orofacial muscles in comparison to trunk and limb muscles. The orofacial muscles include the tongue muscles and the branchiomeric muscles in the lower face. Their main functions are chewing, swallowing, and speech. All orofacial muscles originate from the mesoderm of the pharyngeal arches under the control of cranial neural crest cells. Research in vertebrate models indicates that the molecular regulation of orofacial muscle development is different from that of trunk and limb muscles. In addition, the regenerative ability of orofacial muscles is lower, and they develop more fibrosis than other skeletal muscles. Therefore, specific approaches need to be developed to stimulate orofacial muscle regeneration. Regeneration may be stimulated by growth factors such fibroblast growth factors and hepatocyte growth factor, while fibrosis may be reduced by targeting the transforming growth factor β1 (TGFβ1)/myofibroblast axis. New approaches that combine these 2 aspects will improve the surgical treatment of orofacial muscle defects.

Keywords: satellite cells, skeletal muscle, growth factors, cleft palate, tissue regeneration, fibrosis

Introduction

Orofacial clefts and orofacial trauma are clinical conditions that involve impaired orofacial muscle regeneration as well as fibrosis. Orofacial clefts are common birth defects that often include cleft palate and/or a cleft in the soft palate in about 46% of the cases (Andersson et al. 2010; Wehby and Cassell 2010). These disorders occur in 1:500 to 1:2,500 births and are surgically treated in the first or second year after birth. Prior to reconstructive surgery, these children show impaired speech development and swallowing. After surgery, speech problems persist in about 30% of the patients, which may lead to social and psychological problems. This is mainly caused by incomplete muscle regeneration and fibrosis (Carvajal Monroy et al. 2012).

The orofacial muscles include the muscles of mastication, the muscles of cheeks and lips, the soft palate muscles, the suprahyoid muscles, and the tongue muscles (Le Reverend et al. 2014). These muscles develop from the mesoderm of the pharyngeal arches and the occipital somites (Sugii et al. 2017; Chang and Kioussi 2018). The muscles of the trunk and limbs develop from the thoracic and lumbar somites, respectively (Chang and Kioussi 2018). The molecular pathways that regulate orofacial muscle development differ from those in trunk and limb muscles (Chang and Kioussi 2018). Embryonic myogenesis in skeletal muscles involves the differentiation of mesodermal cells into myogenic cells. These cells migrate to their specific body region and differentiate into myoblasts, proliferate, and fuse into myofibers (Buckingham 2017). Another population of precursor cells will later become the quiescent satellite cells (SCs) in the adult muscle that provide muscle growth and regeneration after injury, although their origin is still controversial (Ono et al. 2010). Upon injury, regulatory pathways are activated that also function during muscle development (Hernandez-Hernandez et al. 2017).

The functions of human orofacial muscles include chewing, swallowing, and speech. Chewing is carried out by movements of the lower jaw (Le Reverend et al. 2014) while swallowing requires the coordinated action of the tongue, soft palate, and the suprahyoid muscles (Le Reverend et al. 2014; Harandi et al. 2017). Speech is coordinated by contractions of the orofacial muscles and the larynx (Harandi et al. 2017). The functioning of muscle fibers is determined by specific myosin...
heavy-chain (MyHC) isoforms (Schiaffino et al. 2015). During development, myofibers express embryonic MyHC, which is gradually replaced by other isoforms (Schiaffino et al. 2015). The exact regulation of expression of specific MyHC isoforms is still unclear.

In addition to the developmental differences, orofacial muscles seem to regenerate less and develop more fibrosis after injury than limb and trunk muscles (Pavlath et al. 1998; Carvajal Monroy et al. 2015; Cheng et al. 2018). Thus, specific approaches are required to improve orofacial muscle regeneration after surgical reconstruction. Here we discuss orofacial muscle development and regeneration to identify specific targets to improve the treatment of orofacial muscle defects.

Orofacial Muscle Development

The orofacial muscles comprise the muscles of the lower face and the oral cavity. Their development takes place in 2 different regions of the embryo head (Chang and Kioussi 2018). The mesoderm of the pharyngeal arches gives rise to the branchiomeric muscles that include the masticatory muscles, the buccinator, the orbicularis oris muscles, the muscles of the soft palate, and the suprathyroid muscles (Fig. 1). The occipital somites give rise to the muscles of the tongue (Fig. 1D) (Michailovici et al. 2015; Chang and Kioussi 2018; Schubert et al. 2018). Studies in chicks, rodents, and zebrafish show that cranial neural crest cells (CNCCs) in the embryo head direct muscle development and differentiate into the intramuscular connective tissue (Tzahor et al. 2003; Grenier et al. 2009; McGurk et al. 2017). In zebrafish and other vertebrates, retinoic acid (RA) maintains the undifferentiated state of precursor cells during proliferation (El Haddad et al. 2017; McGurk et al. 2017). Subsequently, the degradation of RA allows the precursor cells to form myofibers and tendons (McGurk et al. 2017). The main regulatory factors and pathways are discussed in the next sections.

Molecular Regulation of Branchiomeric Muscle Development

Cranial Neural Crest Cells and Myogenic Commitment. The branchiomeric muscles (Fig. 1) develop from the first, second, and fourth pharyngeal arches (Chang and Kioussi 2018). The third pharyngeal arch gives rise to the stylopharyngeous muscle, which is not considered an orofacial muscle (Frisdal and Trainor 2014). In the developing neural tube, embryonic cells in the margins of the neural folds undergo epithelial-mesenchymal transition (EMT) and form the neural crest (Scarpa and Mayor 2016; Szabo and Mayor 2018). Cells along the neural crest delaminate and migrate toward the different regions in the embryo (Scarpa and Mayor 2016; Szabo and Mayor 2018). Some CNCCs migrate to the pharyngeal arches, where they form the intramuscular connective tissue and regulate orofacial muscle development (Grenier et al. 2009; Scarpa and Mayor 2016). Their migration is stimulated by autocrine growth factors such as stromal-derived growth factor (SDF) and vascular endothelial growth factor (VEGF) (Scarpa and Mayor 2016). SDF and VEGF are also produced during muscle regeneration after injury, which requires the migration of myoblasts (Von den Hoff et al. 2019).

In the pharyngeal arches of zebrafish, the proliferation of CNCCs is regulated by platelet-derived growth factors and Talin 1, which connects the cytoplasmic tails of integrins to the actin cytoskeleton via vinculin (McCarthy et al. 2016; Ishii et al. 2018). After proliferation, the CNCCs induce the concentration of mesodermal cells in the core of the pharyngeal arches (Fig. 2). In mouse embryos, both mesodermal cells and CNCCs express paired-like homeodomain transcription factor 2 (Pitx2) (Shih et al. 2007). Pitx2 knockout mouse embryos show reduced proliferation of both cell types, smaller muscles, and reduced expression of T-box transcription factor 1 (Tbx1) in mesodermal cells and CNCCs (Shih et al. 2007; Harel et al. 2012). In vitro, C2C12 cells overexpressing Pitx2 express increased levels of Tbx1 (Shih et al. 2007). These data indicate that PITX2 induces TBX1 to regulate the proliferation and concentration of mesodermal cells in the core of the pharyngeal arches surrounded by CNCCs (Grenier et al. 2009; Kong et al. 2014). Interestingly, Tbx1 knockout mouse embryos lack the core mesodermal cells (Grenier et al. 2009; Kong et al. 2014).
The CNCCs then differentiate into connective tissue precursors, while the mesodermal cells commit to the myogenic lineage (Fig. 2). Tbx1 knockout mouse embryos show reduced expressions of transcription factors such as scleraxis (SCX) in the CNCCs and capsulin (TCF21), myogenic repressor (MYOR), and myogenic factor 5 (MYF5) in the mesodermal cells (Shih et al. 2007; Grifone et al. 2008; Grenier et al. 2009; Moncaut et al. 2012; Kong et al. 2014). In these embryos, tendon and muscle formation is impaired. Tcf21 and MyoR double-knockout mouse embryos showed reduced MYF5 expression in the core mesoderm and impaired muscle formation (Moncaut et al. 2012). Thus, the connective tissue precursor cells express Scx while committed myogenic cells express Myf5. SCX-positive precursors proliferate and invade the mesodermal core to induce the differentiation of the committed cells into myoblasts. In Tbx1 knockout mouse embryos, both the expression of Scx by precursor cells and the number of myoblasts are reduced (Grifone et al. 2008; Grenier et al. 2009; Kong et al. 2014). This also indicates that TBX1 is crucial for myogenesis in the branchiomipheric muscles. To summarize, CNCCs induce the concentration of mesodermal cells in the core of the pharyngeal arches. CNCCs then differentiate into SCX-positive precursors and the mesodermal cells commit to myogenesis. Subsequently, the SCX-positive cells induce the early differentiation of the committed cells into myoblasts. This is a crucial interaction between the neuroectoderm and the mesoderm during orofacial myogenesis.

**Myofiber Formation.** The final steps in branchiomipheric muscle formation are the differentiation of myoblasts into myofibers and the formation of the intramuscular connective tissue and the tendons (Fig. 2). In chick embryos, SCX-positive precursors also express bone morphogenetic protein 4 and 7 (BMP4 and BMP7), WNT1, WNT3a, noggin, gremlin, and frizzled-related protein 3 (FRZB) during proliferation (Tzahor et al. 2003). In vitro, BMP and WNT inhibit the proliferation and differentiation of myoblasts, which can be rescued by the addition of noggin, gremlin, or FRZB (Tzahor et al. 2003). Simultaneously, Dlx5 is expressed by SCX-positive precursors (Sugii et al. 2017). Dlx5 knockout mouse embryos in this study show reduced fibroblast growth factor 10 (FGF10) expression and impaired branchiomipheric muscle formation. In cultured myogenic cells from these knockout, myofiber formation is rescued by FGF10 (Sugii et al. 2017). This suggests that FGF10 produced by SCX-positive precursors regulates the differentiation of myofibers by inducing myogenic regulatory factors (MRFs; see Table). In zebrafish, Scx-positive precursor cells express cytochrome P450-26B1 (cyp26b1), which degrades RA and thus promotes the differentiation of SCX-positive precursors in the pharyngeal arches (McGurk et al. 2017). The precursors differentiate to form the tendons and the intramuscular connective tissue (McGurk et al. 2017). This indicates a neuroectodermal origin of the muscular connective tissue in branchiomipheric muscles. This aspect will be discussed in the regeneration section of this review.

During myofiber formation, the MYF5-positive myoblasts express paired homebox factor 7 (Pax7) and myogenic differentiation factor (MyoD) (Grifone et al. 2008; Moncaut et al. 2012). Myf5 and MyoD double-knockout embryos lack differentiated branchiomipheric muscles (Kablar et al. 2003). Normally, MYOD-positive cells start to express myogenin (MyoG), fuse, and form myofibers (Hernandez-Hernandez et al. 2017; Chang and Kiousi 2018) (Fig. 2). Myoblasts also express Notch during myofiber formation, while Notch knockout embryos show small or absent branchiomipheric muscles (Czajkowski et al. 2014). In vivo, PAX7- and MYOD-positive myoblasts overexpressing Notch do not form myotubes but remain in the proliferation phase (Mourikis et al. 2012). Within myofibers, satellite cells (SCs) reside between the sarcolemma and the basal lamina in a quiescent state (Mourikis et al. 2012; Czajkowski et al. 2014). In vivo and in vitro studies show that NOTCH maintains the pool of SCs throughout life (Mourikis et al. 2012; Czajkowski et al. 2014). As in trunk and limb muscles, the SCs can regenerate orofacial muscle tissue, which will be discussed later.

**Molecular Regulation of Tongue Muscle Development**

**Occipital Somites, Cell Migration, and Myogenic Commitment.** The development of the tongue muscles occurs in the occipital somites and the mesoderm of the tongue bud (Fig. 3).
Mesodermal cells commit to myogenesis in the occipital somites, which is induced by CNCCs (Fig. 3–1). These CNCCs have migrated from the neural crest region toward the occipital somites (Noden et al. 1999; Szabo and Mayor 2018). There, the early myogenic differentiation of mesodermal cells occurs. Cultured somites of chick embryo heads express \(\text{MRFs} \) in the mesodermal cells upon addition of sonic hedgehog (SHH), Wnt1, or Wnt3 (Munsterberg et al. 1995; Noden et al. 1999). In the occipital somites of mouse embryos, \(\text{Bmp4, Wnt3a, Wnt3b, and Shh} \) are expressed by CNCCs, while \(\text{Mrf5} \) are expressed by the mesodermal cells (Tzahor et al. 2003). The factors that stimulate the expression of these proteins are not known.

After commitment, the myogenic cells from the occipital somites migrate toward the forming tongue and differentiate into myoblasts (Fig. 3–2). Chick embryo studies indicate that \(\text{PAX3- and PAX7-positive myogenic cells migrate along the hypoglossal nerve} \) (Huang et al. 1999; Buckingham 2017). The tongue bud is growing by the proliferation of mesodermal cells and the invading CNCCs in the midline of the first pharyngeal arch (Han et al. 2014; Du et al. 2016; Szabo and Mayor 2018). In the developing tongue bud of mouse embryos, CNCCs start to express \(\text{Scx} \) and turn into connective tissue precursors (Han et al. 2014). \(\text{Scx} \) expression by CNCC-derived precursors is induced by transforming growth factor \(\beta1 \) (TGF\(\beta1\)) and WNT1, as shown in studies on double-knockout mouse embryos (Hosokawa et al. 2010). These embryos exhibit microglossia and disorganized myofibers. \(\text{Wnt3a, Fgf1, Fgf2, Fgf5, and Fgf6} \) are expressed in the tongue mesoderm of mouse embryos and seem to induce \(\text{Myf5, Pax7, and MyoD} \) (Han et al. 2014; Du et al. 2016). However, \(\text{Myf5} \) knockout mouse embryos show reduced levels of FGF6 and an atrophic tongue (Han et al. 2012). In vitro, myogenesis in the tongue bud of these knockouts can be rescued by FGF6 (Han et al. 2012). These data indicate that FGFs induce myogenesis in the forming tongue, while there seems to be a positive feedback by MRFs.

**Myofiber Formation.** Finally, myofibers and connective tissue are formed in the tongue bud (Fig. 3–3). Organ cultures of mouse embryo tongues show that \(\text{Mrfs} \) expression and myofiber formation require BMP- and SMAD-dependent Tgfb1 expression by SCX-positive precursors (Han et al. 2012). \(\text{Myf5-Smad} \) double-knockout mouse embryos show continued myoblast proliferation, reduced Myog expression, and low myofiber formation, leading to an atrophic tongue. In wild-type tongue buds, SCX-positive precursors surround the myogenic cells and induce their differentiation (Han et al. 2012). As in branchiomeric muscles, NOTCH represses myofiber formation and maintains the pool of postnatal SCs (Czajkowski et al. 2014).
myofibers and the connective tissue allows complex tongue movements in speaking and deglutition. The interaction between the neuroectodermal connective tissue and the pharyngeal mesoderm is also crucial during tongue myogenesis. Similar interactions might influence muscle regeneration after injury. This will be discussed in the next sections of this review.

Genetic Factors in Orofacial Clefts and Muscle Development

Orofacial clefts (OFCs) are classified into syndromic and nonsyndromic cases. The nonsyndromic cases represent about 70% of all OFC cases (Beaty et al. 2016; Adeyemo and Butali 2017). Genes such as PAX7, GRHL3, FGF, FGFR2, IRF6, BMP4, TGFα, TGFβ1, and PAX1 are reported as causal genes for OFC (Beaty et al. 2016; Li et al. 2016; Adeyemo and Butali 2017). Of these genes, PAX7, BMP4, FGF2, and FGF5 are also related to muscle development. In vertebrates, PAX7 is involved in the regulation of CNCC migration, and at later stages, it is also expressed by myogenic cells and myoblasts (Leslie et al. 2015; Buckingham 2017). Postnatally, PAX7 in SCs regulates muscle regeneration after injury (Leslie et al. 2015; Buckingham 2017). In mouse embryos, BMP4 induces the proliferation of myogenic cells during tongue, lip, and soft palate development but not their differentiation (Han et al. 2014; Leslie et al. 2015; Saket et al. 2016). In contrast, FGF2 and FGF5 only stimulate the differentiation of myogenic cells into myoblasts and their fusion into myofibers (Han et al. 2012; Li et al. 2016). Although there is no direct evidence, these genes might be related to impaired muscle development and regeneration in OFC patients.

Differences with Trunk and Limb Muscles during Development

In trunk and limb muscles, the regulation of myogenesis is quite different. Several excellent reviews discuss this in detail, but we will give a short overview here (Bismuth and Relaix 2010; Michailovici et al. 2015; Hernandez-Torres et al. 2017; Nassari et al. 2017; Chang and Kioussi 2018; Schubert et al. 2018). In the thoracic and lumbar somites, the committed myogenic cells migrate under the control of WNT1, WNT3, WNT7a, and sonic hedgehog (SHH) expressed in the notochord and the neural tube (Chang and Kioussi 2018). In contrast to the branchiomeric muscles, FRZB, gremlin, and noggin do not seem to play a role in myogenesis. In trunk and limbs, the mesoderm differentiates into the tendons and the intramuscular connective tissue while the migrated committed cells differentiate into myoblasts (Nassari et al. 2017). In trunk and limb myogenesis, SIX1 and SIX4 induce PAX3, which stimulates the expression of MRFs by myoblasts (Bismuth and Relaix 2010). The MRFs induce the proliferation and differentiation of myoblasts into myofibers. PAX3 also stimulates the expression of PITX2, which induces PAX7 and MYOD to regulate myofiber formation (Bismuth and Relaix 2010; Hernandez-Torres et al. 2017). Thus, orofacial myogenesis differs from that in other skeletal muscles by the molecular interactions between CNCCs and mesodermal cells.

The Origins of Satellite Cells

In the branchiomeric muscles of chick embryos, SCs are present in the core of the pharyngeal arches together with myogenic cells (Harel et al. 2009). The SCs remain quiescent during myofiber formation. This suggests that branchiomeric SCs and myogenic cells share a common mesodermal origin. In mouse embryos, SCs are also present in the occipital somites (Harel et al. 2009). These SCs migrate to the first pharyngeal arch together with myogenic cells (Harel et al. 2009). In trunk and limb muscles, mesodermal cells in the somites give rise to both the myogenic cells and the SCs (Aziz et al. 2012; Yin et al. 2013). Both cell types migrate toward specific body regions where the myogenic cells differentiate into myofibers (Aziz et al. 2012; Yin et al. 2013). The SCs reside at the outer
The activity of SCs largely depends on their microenvironment or niche (Grefte et al. 2007; Ten Broek et al. 2010; Hernandez-Hernandez et al. 2017) (Fig. 4). Their activation takes place by growth factors such as FGF6, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) (Ten Broek et al. 2010). FGF1 and FGF2 secreted by SCs after injury stimulate their own proliferation and the expression of MRFs (Pawlikowski et al. 2017). In addition, both SCs and invading macrophages secrete matrix metalloproteinases (MMP2 and MMP9) to degrade extracellular matrix (ECM) components. Fibroblasts differentiate into myofibroblasts stimulated by TGFβ1 to deposit ECM components, mainly collagen I. TGFβ1 is the main factor inducing fibrosis and reducing SC proliferation and myofiber formation in all adult muscles (Yin et al. 2013; Wynn and Vannella 2016). As discussed earlier, during tongue development, TGFβ1 promotes myofiber formation (Han et al. 2012). Clearly, the role of TGFβ1 is different in muscle development and in the adult muscle.

In mice, freeze injuries of the masseter muscle regenerate less effectively than similar injuries of the tibialis anterior muscle (Pavlath et al. 1998). This was shown by a lower number of regenerated myofibers of smaller diameter. Moreover, much more fibrous tissue was formed. In a model for muscle regeneration in the rat, a circular full-thickness wound was made in the soft palate (Carvajal Monroy et al. 2013; Carvajal Monroy et al. 2015). Large numbers of myofibroblasts were detected in the wound area around 7 d after wounding. Histology also showed a 3-fold increase in collagen content at 56 d, while only few myofibers had regenerated. In vitro, SCs from the masseter muscle of mice show a higher proliferation rate and a delayed differentiation compared with SCs from the extensor digitorum longus muscle (Ono et al. 2010). This was also shown for SCs from other orofacial muscles, including the soft palate muscles in rats (Carvajal Monroy et al. 2015; Carvajal Monroy et al. 2017). Interestingly, when transplanted, mouse masseter-derived SCs regenerated limb muscles as efficiently as those from the EDL itself (Ono et al. 2010). These data indicate that orofacial muscles regenerate less than limb and trunk muscles and develop more fibrosis. Other adult connective tissues derived from the ectoderm such as that in skin and heart also show extensive fibrosis after injury (Parfjejevs et al. 2018). It appears that ectoderm-derived fibroblasts respond more strongly to TGFβ1, readily differentiate into myofibroblasts, and produce ECM components at a higher rate. Therefore, the ectodermal origin of the connective tissue in orofacial muscles might explain why these muscles develop more fibrosis and regenerate less than other skeletal muscles.

**Orofacial Muscle Regeneration**

In all skeletal muscles, regeneration starts with inflammation followed by tissue formation and remodeling, which may end up in fibrosis (Grefte et al. 2007). Injury disrupts the connection between the muscle fibers and the adjacent connective tissue, thereby activating the SCs (Thomas et al. 2015). SCs are the stem cells of muscle tissue and mainly responsible for its regenerative capacity. Proliferating SCs express PAX7 and differentiate into myoblasts that start to express MYOD and then fuse to form MYOG-positive myotubes maturing into myofibers. The activity of SCs largely depends on their microenvironment.
factor (IGF), VEGF, platelet-derived growth factor (PDGF), and stromal cell-derived factor (SDF) (Grefte et al. 2007; Ten Broek et al. 2010; Von den Hoff et al. 2019). These factors are present in the ECM of skeletal muscles and regulate the proliferation and differentiation of SCs (Grefte et al. 2007, Ten Broek et al. 2010). These growth factors might be used to improve the regenerative ability of orofacial SCs. Strategies to promote regeneration in trunk and limb muscles include the inhibition of fibrosis with microRNAs (miRNAs), decorin, and small molecules such as losartan that target the TGFβ1/myofibroblast axis (Garg et al. 2015; Vancheri et al. 2018; Von den Hoff et al. 2019). As discussed earlier, the proliferation and differentiation of ectoderm-derived fibroblasts into myofibroblasts are strongly stimulated by TGFβ1. The implantation of SCs or nonmyogenic stem cells in the site of the injury might not be the best approach since they generally do not survive for long in vivo (Von den Hoff et al. 2019). A more efficient strategy might be the delivery of growth factors and antifibrotic components to the impaired tissue in scaffolds or hydrogels. This might also be more cost-effective for future clinical applications.

Future strategies to promote orofacial muscle regeneration should target the specific aspects of orofacial muscle development and regeneration. Local SCs and other stem cells with myogenic potential may be stimulated by applying growth factors such as FGFs and HGF. Simultaneously, the TGFβ1/myofibroblasts axis should be targeted by using small-molecule inhibitors or miRNAs to prevent fibrosis. This may enhance orofacial muscle regeneration and the treatment of orofacial muscle defects.

**Conclusion**

Orofacial muscle development is regulated by CNCCs that will eventually form the intramuscular connective tissue and the tendons. Thus, the connective tissue of orofacial muscles is of ectodermal origin. In contrast, the development of trunk and limb muscles is induced by the notochord in the thoracic and lumbar somites. In contrast to the CNCCs, the NCs in trunk and limb muscle development do not differentiate into the intramuscular connective tissue. Instead, the connective tissue is of mesodermal origin. In general, connective tissue of ectodermal origin has a strong tendency for fibrosis after injury. Similarly, the ectodermal origin of the connective tissue in orofacial muscles might increase fibrosis. In addition, SCs from orofacial muscles have a lower differentiation capacity compared to those from trunk and limb muscles. Thus, the improvement of orofacial muscle regeneration should therefore focus on both the stimulation of SC differentiation and the reduction of fibrosis. Therapeutic strategies to improve the regeneration of orofacial muscles (e.g., after surgery in orofacial clattering) should include antifibrotic agents as well as growth factors that stimulate satellite cell differentiation such as FGFs and HGF. These approaches may greatly improve the outcome of orofacial cleft surgery and reconstructive surgery after tumor resection or trauma in the orofacial region.

**Author Contributions**

D.H. Rosero Salazar, contributed to conception, drafted the manuscript; P.L. Carvajal Monroy, J.W. Von den Hoff, contributed to conception and design, critically revised the manuscript; F.A.D.T.G. Wagener, contributed to design, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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