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Advances and Challenges in Systemic Stem Cell Therapy for Muscular Dystrophies

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Introduction

Muscular dystrophies (MD) are heterogeneous group of genetic disorders including more than 30 different types [1,2]. They are generally characterized by progressive degeneration and fibrosis of the skeletal muscles. These disorders are mostly due to gene defects in important structural proteins in muscle which lead to muscle tissue damage and eventually partial or complete loss of muscle function with various morbidity or mortality [3]. Though in many cases the defective genes are known, there is no definitive cure so far. Meanwhile gene delivery or stem cell based therapies are promising approaches to correct the gene defect or restore the skeletal muscle tissue with healthy stem cells which provide the correct genetic information to the muscle [4]. However, except finding the right type of stem cells to restore the muscle, one of the major hurdles in stem cell therapy is the route of cell delivery into the dystrophic muscle. Due to complexity of the muscle groups involved in different muscular dystrophies, local cell delivery is not a feasible and practical approach [5-7]. Therefore, ideally the cells needed to be administered systemically to reach multiple involved muscle groups. These editorial overviews common types of muscular dystrophies, different stem cell populations tested for systemic delivery/engraftment into skeletal muscle and outlines key factors and challenges for successful systemic cell delivery, migration and engraftment into skeletal muscle.

Common Muscular Dystrophies

The most common and most severe form of MD in children is the Duchenne Muscular Dystrophy (DMD) which is a recessive x-linked disease and therefore affects mostly males. DMD is caused by mutations in the dystrophin gene which results in the absence of dystrophin protein leading to membrane instability, degeneration and finally loss of the muscle and its gradual replacement by adipose and fibrotic tissue [8,9]. The disease starts of the age of 3-5yrs and muscle weakness is first seen in the upper leg and arms and progresses rapidly into the loss of the movement ability and later to severe cardiopulmonary complications and patient's death in the mid 20'.

Another common form of MD is the **Becker Muscular Dystrophy (BMD)**. It is similar to DMD and characterized by the inherited mutation in dystrophin gene which leads to production of less functional forms of dystrophin. It again affects mostly boys, but this disease progresses less rapid as compared to DMD. The disease develops later with an onset between 10 yrs of old to the adulthood. The weakness is much milder and more variable including being limited to certain muscles such as the quadriceps muscles. Cardiopulmonary complications- as seen in DMD- are not as common in BMD patients. Some patients can be asymptomatic for years or with very slow and mild progression having a nearly normal life span [8,9].

Myotonic Muscular Dystrophy(MMD) is another common (most common adult form of MD) inherited muscle dystrophy disorder caused by a gene defect of DMPK (Type 1) and CNBP (Type 2) leading to a progressive muscle wasting of the voluntary and involuntary muscles. MMD is characterized by prolonged muscle contraction and difficulties to relax after use and affect muscles of the lower leg, arms, neck and face [10,11]. Symptoms can develop from the time of birth, in childhood or in the adulthood. It is often a multisystem disorder involving cardiac, respiratory, ocular and gastrointestinal problems and also can lead to congenital impairment [12].

Emery Dreifuss Muscular Dystrophy(EDMD) is another common slow progressive MD caused by a gene mutation leading to the loss of Emerin and changes of the Lamin A and C. EDMD starts in the mid childhood to second decade of life and is characterized by muscle weakness (mostly shoulders,

upper arms and calf muscles), joint stiffness (contractures in elbows, neck and heels) and cardiac complications such as conduction blocks [13,14].

Other major MDs are the **Congenital (CMD), Facioscapulohumeral (FSHD), Limb girdle (LGMD), Oculopharyngeal (OPMD)** and **Distal Muscular Dystrophy (DD)**. All of these disorders have progressive muscle degeneration and can be developed at birth or later in the adulthood; some of them include other systemic complications including cardiac, respiratory, gastrointestinal and neurological features [1-3]. But they all have one in common; they are inherited, progressive and systemic muscular dystrophies resulting in the dysfunction and atrophy of multiple muscles over time and unfortunately there is no cure for them so far. Thus it is important not only to find the right treatment but to to identify the best delivery aaproach which allows a widespread distribution of the delivered gene vectors or cells into the affected muscle groups in order to warrant a therapeutic outcome.

Stem Cells Tested Systemically for Muscle Repair

So far, various stem cells and muscle progenitors have been tested for cell therapy in MDs. On the top of these lists are muscle adult stem cells and myoblasts. Skeletal muscle stem cells, called **Satellite Cells (SCs)** which reside at the periphery of the muscle fiber were among early stem cells which have been used for cell based therapy for MDs [15,16]. Despite their superior differentiation potential, low migration capacity in the muscle tissue after intramuscular (IM) injection limited their application [5,6,17,18]. Moreover, systemic delivery such as intra-arterial route was also unsuccessful due to their inability to cross the vessel wall into the muscle interstitium.

Another group of stem cells which have been tested for systemic cell delivery are **muscle derived stem cells (MDSCs)**. These cells can be separated from skeletal muscle after enzymatic digestion and serial pre-plating. They express different levels of Sca-1 and CD34 and are able to differentiate into major mesodermal lineages such as blood, bone or muscle [19,20]. Systemic cell delivery of MDSCs using intra-venous (IV) or intra-arterial (IA) routes has been performed to evaluate their possible engraftment into skeletal muscle [21,22]. Interestingly, these cells demonstrated muscle homing ability and adhesion and transmigration into muscle. Moreover, they have the potential to engraft into skeletal muscle and express dystrophin in mouse model for DMD (*mdx* mouse). Furthermore, their engraftment can be improved by muscle injury/ damage after cell delivery. However, the engraftment efficiency needs to be improved to provide a significant therapeutic outcome.

Muscle **side population cells (SP cells)** are another group of muscle stem cells which have been tested for systemic cell therapy in mice [23]. These cells can be purified by cell sorter based on their efficient efflux of vital DNA dye Hoescht 33342. When transplanted using intra-arterial (IA) route through femoral artery, these cells were able to engraft into perfused muscles and differentiate into mature fibers [24]. However, the engraftment efficiency was below therapeutic level.

Another group of muscle stem cells tested for systemic delivery into muscle are $CD133^{+}$cells which were initially isolated from peripheral blood [25] and later on from skeletal muscles [26]. Both have been tested for intramuscular and intra-arterial cell delivery. Upon transplantation, they give rise to human myofibers and demonstrate great engraftment after IM injection. However their IA engraftment was at modest levels.

Mesenchymal stem cells (MSCs) were another group of the cells which have been tested for myogenic potential. First studies using MSCs demonstrated limited myogenic differentiation potential in dystrophic mice [27] as the level did not reach therapeutic levels. Later, other groups [28,29] reported a higher level of engraftment using modified human MSCs after intra-muscular (IM) and intra-venous (IV) delivery in mice; however, still functional recovery could not be achieved [30]. Recently another group reported a subpopulation of $CD271^{+}$ MSCs which are able to differentiate efficiently into myotubes by forced MyoD over-expression. Interestingly, these MyoD-induced MSCs were able to engraft after intra-arterial delivery in a dog model for DMD, through the level of dystrophin expression was unfortunately low [31].

Another important group of stem cells tested for muscle repair are **vessel associated pericytes.** Many studies have used human **mesoangioblasts** or **pericytes** as vessel associated progenitors in murine [32,33] or dog models [34] for MDs. These studies demonstrated their efficient engraftment following intra-arterial (IA) delivery in the downstream muscles of affected animal. Based on available data, mesoangioblasts are considered as one of the best cell therapies suitable for systemic delivery in MDs.

Adipose derived stem cells (ADSCs) have also been tested for cell therapy for muscle repair [35,36]. Both studies used intra-venous (IV) delivery and new engrafted muscle fibers and partial functional improvement was observed in treated muscles. Interestingly, cell surface marker expression and their proliferation and differentiation capacity had some similarities to the mesoangioblasts. Furthermore, these cells can be isolated easily in abundance from patients by liposuction which is another advantage for these cells. Further studies needed to validate the efficiency of ADSCs for skeletal muscle repair in MDs following systemic delivery routes.

Human Embryonic (ES) and induced Pluripotent Stem Cells (iPSCs) are other major groups of stem cells which have offered promising opportunities for stem cell based regenerative medicine. In particular, iPS technology has the advantage of avoiding ethical and immunological concerns associated with ES cells as they are directly derived/reprogrammed from patient's somatic cells [37]. Previous studies using murine or human ES/iPS cells have demonstrated high efficiency of myogenic induction using transient PAX3 or PAX7 over-expression [38,39]. Derived myogenic cells were able to engraft and express dystrophin in vivo and seed satellite stem cell pool and improve muscle contractility following IM injection in mice models for MDs. Moreover, in case of murine ES/iPS cells, they demonstrated systemic engraftment potential following IA or IV injection [39,40]. Also, another group [41] have recently derived genetically corrected mesoangioblasts – like cells from patient's iPS cells and demonstrated their engraftment potential in a limb girdle muscle dystrophy (LGMD) mouse model. These cells also demonstrated great myogenic differentiation and improved muscle function after intra-arterial (IA) delivery. Overall, iPS based cell therapies for skeletal muscle disorders is a fast pacing field and more research are being done in order to differentiate the right type of patient- specific muscle stem cells from iPS cells suitable for gene correction and systemic therapy in MDs.

Systemic Cell Delivery Routes to Target Skeletal Muscle

As mentioned above, one of the major drawbacks in cell therapy for MDs is poor cell survival and migration following IM injection as well as multi-compartment muscle involvement in many MDs which makes IM injection not a feasible clinical approach. Therefore in theory, systemic cell delivery provides much better efficiency to reach and target multiple muscle groups and to replace and repair damaged muscle.

Initially intra-venous (IV) route (either the tail or femoral vein) was used for mesoangioblasts delivery into dystrophic mice muscles. However the engraftment efficiency was low and did not reach therapeutic potential [33]. This was mainly due to cell entrapment in other organs (such as lung and spleen barriers) and their dilution before reaching the skeletal muscle.

Therefore in order to bypass this first-pass effect, the intra-arterial (IA) method was considered after first studies showing a better engraftment in the target tissue compared to the IV delivery. For IA delivery, many studies have used femoral artery [33,41,42] for cell delivery into hind limb muscles, while few others used the iliac artery approach [22,43].

Intra-arterial cell delivery using femoral artery cannulation is a well-practiced method which allows proper and uniform perfusion of downstream muscles in animal models. A recent study [44] has investigated time-course kinetic and efficiency of IA (intra-femoral artery) cell delivery and migration into dystrophic mice muscles over 24 hours using nano particle-labeled $CD133^{+}$ cells (from human peripheral blood) combined with micro CT imaging.

The data indicated progressive distribution of the cells from anterior to medial and posterior regions of perfused hind limb in the first 2 hours after IA delivery. Interestingly, serial passage of the cells through circulation was necessary to enhance cell migration into dystrophic muscles from 4% at the time of infusion to a maximum 20% of injected cells 60 minutes later [44].

Major distributions were seen in Quadriceps, Gastrocnemius and rear thigh muscles compared to Tibialis Anterior and Extensor Digitorum Longus. This might be attributed to the higher capillary density in slow-twitch muscles compared to fast-twitch muscles such as later ones (Cho et al. [47]). As expected, cell presence was negligible in the contra-lateral muscle tissue (less than 3% in average). Furthermore, 12% of the injected cells were entrapped in other non-target organs such as spleen, kidney and lung [44].

Overall, based on the available data intra-arterial (IA) cell perfusion in an established and well characterized route which demonstrates uniform and robust cell infusion into downstream skeletal muscles and does not have short-comings associated with IV route such as first-pass liver, lung and spleen barriers.

Important factors in Systemic Stem Cell Delivery efficiency into Skeletal Muscle

Major determining factors in cell engraftment after intra-arterial (IA) delivery can be classified in two groups:

a) Migration capacity from capillaries to the muscle interstitium

b) Cell survival and differentiation potential into myofibers

These factors are demonstrated in Figure 1. At first, infused cells have to adhere to the endothelial wall and pass through endothelium into interstitial space. Several factors are important for this process including the presence of proper adhesion molecules and their ligands on cells and endothelium, the presence of inflammatory cytokines and chemotactic signals from inflamed muscle and their receptors on the cells and finally hemodynamic factors which influence intra-capillary pressure, time for interaction between the cell and endothelium and endothelium pore size and permeability to the cells.

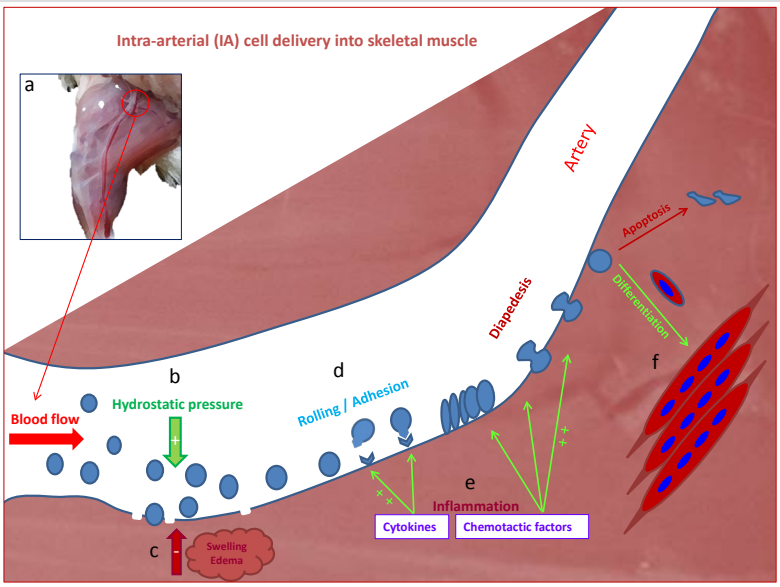

Figure 1- Important factors for intra-arterial stem cell delivery and engraftment into skeletal muscle

Schematic figure demonstrates factors influencing cell delivery and engraftment into skeletal muscle following intra-arterial infusion of the cells.

(**a**) Upper left image demonstrates cannulation site of femoral artery in mouse at proximal point to ensure complete hindlimb muscle perfusion. As discussed in the article, appropriate balance between volume and hydrostatic pressure (**b**) helps cell perfusion and migration while avoids tissue swelling and edema (**c**). Expression of right ligands and receptors for adhesion and diapedesis of the cells (**d**) and the presence of appropriate inflammatory cytokines and chemotactic factors (**e**) in inflamed muscle and their receptors on cells facilitates stem cell migration into muscle interstitium and their final maturation into myofibers (**f**).

Previous studies using MDSCs and CD133 cells have identified few of important adhesion molecules governing adhesion, rolling and diapedesis of the cells [21,43]. Among these molecules, the expression of VCAM-1 on endothelium is crucial as it facilitates adhesion of the infused cells through its ligand VLA-4 (CD49d, CD29 dimer). Therefore both endothelium and infused cells should express appropriate receptors and ligand in order to support proper adhesion and subsequent diapedesis of the cells through endothelium. Interestingly, inflammation and exercise of the dystrophic muscle prior to cell infusion can significantly increase the expression of VCAM-1 on muscle endothelial capillary network. Other important factors which might affect the entire process of adhesion and rolling of the cells are LFA-1 (ICAM-1 ligand), CD44, PSGL (E-selectin ligand) and L-selection (receptor for MadCAM-1).

Another important factor in the migration efficiency of transplanted cells is the presence of inflammatory cytokines in the target muscle tissue before cell infusion. Generally in the case of muscular dystrophies, the environment of the dystrophic muscle is already inflamed by the presence of certain growth factors, inflammatory and chemotactic cytokines such as TNFα, FGF, HGF, SDF-1 and its receptor CXCXR4, oxidative stress and immune cells. Indeed, many of these factors provide chemotactic signaling for attracting inflammatory cells to the muscle.

This chemotactic condition can also be used for attracting infused stem cells to repair the inflamed muscles by inducing inflammation or mild injury in the muscle through exercise which facilitates cell migration and engraftment, as it has been demonstrated in mice models using swimming exercise [22].

Another example is nitric oxide pre-treatment which enhanced mesoangioblasts migration capacity into muscle due to chemotactic response to the growth factors and protected them from apoptosis [45]. Also pre-treatment of mesoangioblasts with TNF-α and SDF-1 factor improved their migration into the interstitium due to the expression of surface molecules important for cell adhesion and migration [46]. Furthermore, transfection of the cells with L-selectin and α4-integrin increased the engraftment level by threefold. More importantly, these pre-treatments did not change the myogenic differentiation of the infused cells while enhanced their rolling and adhesion capacity to the vessel wall.

Lastly hemodynamic factors also play a very important role in IA cell delivery outcome. These can be affected through changing infusion volume, rate of infusion, cell density, vascular tone, hydrostatic pressure and the presence of swelling and edema in inflamed muscle. The comparison of different volumes in mice studies has indicated that the using larger volume for IA infusion improves gene delivery into muscle [47]. The reason is that, first a larger endothelial surface area will be targeted before dilution in blood and second, the permeability is increased due to the higher pressure allowing a better migration into the tissue. In addition, pre-treatment of vasoactive agents such as histamine can increase the permeability of the capillaries in the skeletal muscle [48].

On the other side, the pressure in the muscle tissue induced by inflammation and intra-arterial infusion plays a crucial and may be a contradicting role. Obviously higher pressure will increase cell migration due to the increased hydrostatic pressure which forces the cells into the interstitium. However, increased bulk fluid flow into the muscle tissue will eventually lead to muscle swelling and edema and consequently a reduced infusion due to the compression and eventual collapse of capillaries [47,49]. Therefore appropriate balance and fine tuning between volume and rate of infusion and hydrostatic pressure is essential to ensure proper tissue perfusion with minimal tissue swelling and capillary collapse.

Concluding Remarks

So far, different stem cells including mesoangioblasts, pericytes, MSCs, MDSCs, SP cells and ES/iPS derived cells have been tested for systemic stem cell therapy in animal models of muscular dystrophies. Available data from these studies suggests intra-arterial (IA) delivery as a potential systemic cell delivery route in these cases, though the engraftment efficiency appears to be very variable and in many cases below the therapeutic range.

Since multiple parameters are involved in cell delivery, adhesion, diapedesis and engraftment, this goal cannot be achieved without a detailed adjustment of the following parameters: a) Conditioning the environment of the recipient dystrophic muscle through inflammatory cytokines to improve cell adhesion and migration b) donor cell selection and modification based on expression of important adhesion molecules c) pre-treatment of the cells and capabilities with cytokines and vasodilators before infusion and finally d) accurate adjustment of hemodynamics through volume and flow rate control are key factors for a successful stem cell delivery into dystrophic muscle.

In addition, using different arteries in upper regions is another way to improve cell delivery to larger skeletal muscle groups esp. in case of systemic dystrophies. Finally evaluation of cell entrapment and their long-term side-effects in off-target organs is another important safety issue which needs to be addressed appropriately. Therefore, further studies are warranted to investigate the consideration of all these different factors in order to find the best way of systemic stem cell delivery into skeletal muscle with superior therapeutic outcome and minimal side-effects.

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