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Review Article

A Review on Duchenne Muscular Dystrophy - Market Analysis, Existing Products, Clinical Trials, and Preclinical Tissue Engineering Treatments

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Abstract

Duchenne Muscular Dystrophy (DMD) is a severe genetic disorder characterized by progressive muscle degeneration and weakness, primarily affecting young males. Despite extensive research, no cure currently exists for DMD. With a large and expanding DMD treatment market, we expect new therapeutics to take over existing gold standards of therapy and transform patients' prognoses. Existing therapies such as glucocorticoids, physical therapy, cardiac management, and respiratory therapy focus on symptom management and slowing disease progression. Still, there remains a high demand for long-term, effective treatments. Emerging DMD therapies have shown promising results, including gene therapy, exon skipping, and cell transplantation. Additionally, preclinical research in tissue engineering offers the potential for integrating therapeutic cells and bioactive molecules into DMD treatment. However, this field still has many challenges. This review provides an in-depth examination of DMD, including disease pathology, market size and trends, current therapeutic options, and products in clinical trials. We also explore innovative tissue engineering treatments in preclinical stages.

Keywords: Duchenne Muscular Dystrophy; Dystrophin; Satellite Cells; Gene Transfer; Exon Skipping; Cell Transplantation; Tissue Engineering

Abbreviations

Duchenne Muscular Dystrophy (DMD), extracellular matrix (ECM), dystrophin-associated protein complex (DAPC), satellite cell (SC), vascular endothelial growth factor (VEGF), compound annual growth rate (CAGR), antisense oligonucleotides (AONs), adeno-associated virus serotype-9 (AAV9), European Medicines Agency (EMA), Dystrophin Expressing Chimeric (DEC), decellularized extracellular matrix (dECM), hyaluronic acid-photoinitiator (HA-PI), poly(ethylene oxide) (PEO), polycaprolactone (PCL), poly-L-lactic acid (PLLA), insulin-like growth factor 1 (IGF-1), polyethylene glycol (PEG), green fluorescent protein (GFP), muscle progenitor cells (MPCs), hepatocyte growth factor (HGF), polyethylene glycol-fibrinogen (PF), poly(lactic-co-glycolic acid) (PLGA), gelatin–poly(ethylene glycol)–tyramine (GPT), human adipose-derived stem cells (h-ADSCs), basic fibroblast growth factor (bFGF), gingival mesenchymal stem cells (GMSCs)

Introduction

Muscular dystrophy encompasses a group of genetic muscle diseases [1]. In particular, Duchenne Muscular Dystrophy (DMD) is a prevalent muscular disease, which occurs during early childhood and is one of the most severe forms of inherited muscular dystrophy

[1]. DMD is caused by an X-linked recessive gene mutation, found to primarily affect males [1]. DMD has an incidence rate of approximately 1 in 3500 male infants, and is a leading genetic killer in young boys worldwide [2]. DMD is characterized by progressive striated muscle degeneration and weakness, primarily in the skeletal and cardiac muscle [3,4]. This disease is due to a mutation in the dystrophin gene, leading to inadequate and faulty dystrophin protein in the patient [3,4]. Initially, affected patients develop weakness and atrophy in the proximal trunk muscles, including upper arms and legs [5]. As the DMD symptoms progress, they spread to the lower legs, forearms, neck, and trunk [5]. Additional secondary complications commonly arise as the disease progresses, including scoliosis, abnormal joint fixations, deformity, cardiomyopathy, respiratory infections, and dysmotility in the gastrointestinal system [5].

Currently, no direct cure for DMD exists [6]. Instead, the available therapeutic options act to alleviate and control symptoms, slow disease progression, and stabilize associated conditions [6]. Therefore, the market demand for long-term treatment for young patients struggling with DMD is prevalent [7]. Of particular interest, in addition to the expanding application of treatments such as gene therapy, exon skipping, and cell transplantation, increasing preclinical research is being supported for the designing of tissue engineering products to integrate cells and bioactive molecules into the patient for therapeutic applications [7]. In this review, we will compare healthy to diseased muscle tissue, analyze the current DMD market size and trends, investigate the existing therapeutics on the market and the products within clinical trials, and look into some of the novel tissue engineering treatments in the preclinical stage.

Healthy vs diseased muscle tissue

Healthy muscle tissue

Healthy striated muscles, mainly skeletal and cardiac, consist of highly organized muscle tissue made up of fascicles [8]. Fascicles comprise bundles of myofibers (muscle cells) that enable contraction, force generation, and movement [8]. Each myofiber contains sarcomeres, the basic muscle functional units that consist of thin actin filaments and thick myosin filaments responsible for generating contractile force [8]. The bundles of myofibrils are surrounded by an extracellular matrix (ECM) and supported by a cytoskeleton, which acts as a scaffold for sarcomeres, contributing to muscle plasticity and preventing damage from repeated contraction and relaxation [8].

Dystrophin, a substantial protein, plays a pivotal role in maintaining the synchrony of muscle stretching and contraction [8]. It achieves this by anchoring the sarcomere to the ECM [8]. Dystrophin is a key component of the dystrophin-associated protein complex (DAPC), which links cytoskeletal proteins and sarcomere to the ECM, forming the structural unit of muscle tissue (Figure 1) [8,9]. In healthy muscle tissue, the DAPC stabilizes the plasma membrane of striated muscle cells during contraction, preventing contractile-induced damage [8-10].

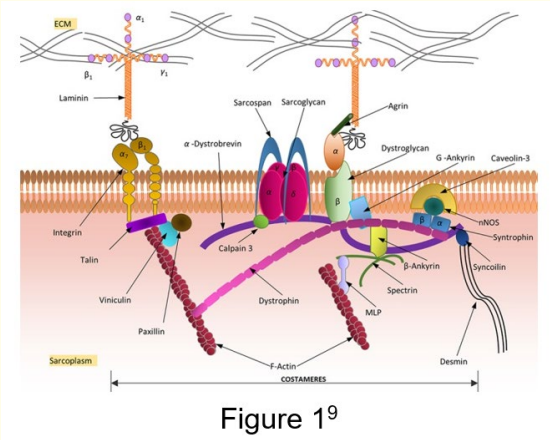


Figure 1: Diagram of the dystrophin-associated protein complex and the main cytoskeletal proteins of healthy skeletal muscle [9].

The image shows dystrophin as a key component of the dystrophin-associated protein complex (DAPC), which links cytoskeletal proteins and sarcomere to the ECM, forming the structural unit of muscle tissue.

Healthy muscle tissue also has the ability to regenerate due to interactions between the adult muscle stem cells, or satellite cells, and the surrounding microenvironment [8]. Satellite cells remain dormant until an injury occurs [8]. Upon muscle injury, chemoattractant growth factors, such as VEGF, fibroblast growth factors, and insulin growth factors, are released by damaged resident cells and inflammatory cells [8]. These growth factors activate the satellite cells, promoting them to migrate to the injury site, where they proliferate and differentiate into muscle fibers for muscle regeneration [8].

Diseased duchenne muscular dystrophy tissue

In DMD patients, mutations in the dystrophin gene result in a complete or near-complete lack of dystrophin protein production [11]. Without functional dystrophin protein, the DAPC is not properly formed, leading to a compromised myofiber membrane [11]. This results in a fragile and permeable membrane, disruption of calcium homeostasis, immune cell infiltration, and oxidative damage [11]. These issues increase the muscle tissue's susceptibility to damage, leading to repeated cycles of muscle cell death and regeneration [11]. Over time, the regenerative capacity of muscle satellite cells in DMD patients becomes exhausted, and fibrous connective tissue and adipose tissue begin to replace muscle fibers as the disease progresses [11]. Figure 2 displays a side-by-side comparison of healthy and DMD muscle tissue [12].

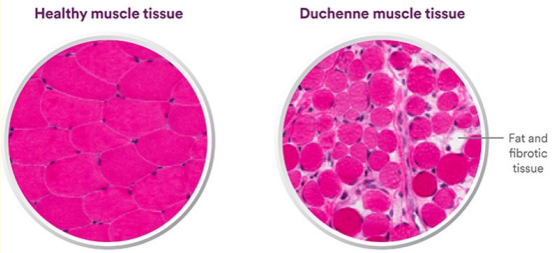


Figure 2[12]

Figure 2: Healthy muscle tissue vs Duchenne Muscle Tissue [12].

The image shows a visual comparing healthy muscle tissue and Duchenne Muscular Dystrophy tissue.

Market size, products, and companies

DMD market size and trends

DMD is one of the most common and severe hereditary neuromuscular diseases, impacting approximately 1 in 3500 male infants worldwide [5]. Approximately 250,000 individuals are affected by muscular dystrophy in the United States [2,5]. DMD patients are primarily diagnosed between 3-5 years old and have a median expected lifespan of 22 years [13]. Figure 3 demonstrates a Kaplan-Meier survival curve for DMD patients, illustrating survival probabilities of 99.8%, 59.5%, 26.1%, and 13.3% at 10, 20, 30, and 40 years, respectively [13].

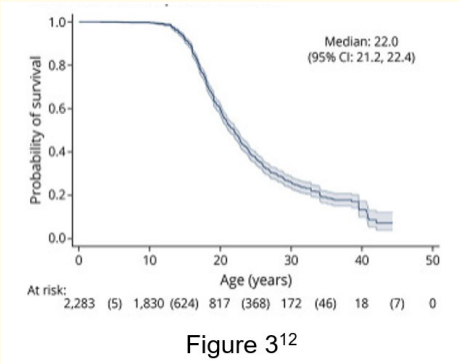
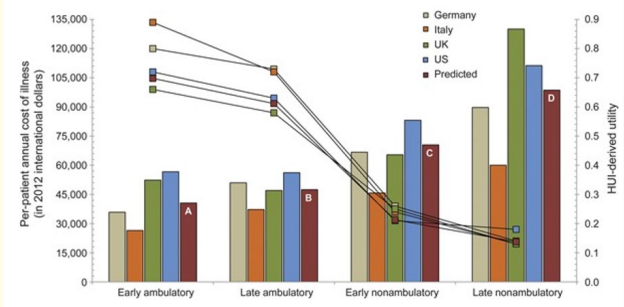


Figure 3[12]

Figure 3: Kaplan-Meier Survival Curve for DMD patients from 14 pooled studies [12].

The image shows a Kaplan-Meier survival curve for DMD patients of age (years) vs probability of survival, illustrating survival probabilities of 99.8%, 59.5%, 26.1%, and 13.3% at 10, 20, 30, and 40 years, respectively.

As DMD patients age, more and worse symptoms and secondary issues present, such as cardiac and respiratory failure, thus increasing the cost of healthcare expenditures [14]. According to a 2014 study by Landfeldt., *et al*. the average annual direct cost of illness per patient ranged from $23,920 to $54,270 (in 2012 international dollars) [15]. The total societal burden per patient was estimated to be between $80,120 and $120,910 [15]. As seen in Figure 4a, there is an increasing trend in the per-patient annual cost as the patient's ambulatory state progressively worsens [15]. This is similarly depicted in Figure 4b as we also see an increase in the annual cost per patient as they age, comparing 0-13 years of age to the 14-29 years of age [15,16].



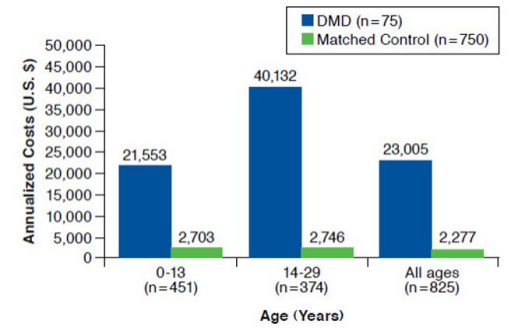


Figure 4b[15]

Figure 4: Mean per-patient annual cost of DMD [14,15].

a) Mean per-patient annual cost of illness (columns) and mean proxy-assessed patient utility (connected markers), by country and ambulatory class.14 b) Average total healthcare costs for DMD and matched control cohorts [15].

According to Mordor Intelligence, the DMD treatment market size was estimated at 2.56 billion USD in 2024 and is projected to reach 4 [32]. billion USD by 2029, with a compound annual growth rate (CAGR) of 11.10% (Figure 5a) [7]. Business Wire predicted a higher trending global market, projecting the market for DMD medication to grow from USD 2 billion in 2022 to USD 27.4 billion by 2030, with a predicted global CAGR of 39.1% between 2022-2030; particularly with China having the largest CAGR of 46%, Japan with a 32.6% CAGR, Canada with a 31.7% CAGR, and Europe and Germany with a 34.8% CAGR [17]. Figure 5b demonstrates geographically where the DMD treatment market is expected to see the greatest growth rate [7].

Figure 5a[7]

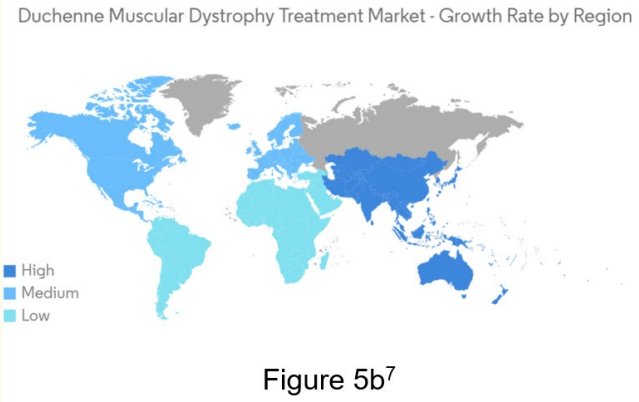


Figure 5b[7]

Figure 5: Schematic of DMD Treatment Market [7].

a) DMD treatment market size based on 2019-2029 study period [7]. b) Growth rate of DMD treatment by region [7].

The DMD market is expected to grow due to new product innovations, high healthcare expenditures, government awareness programs, and an increase in global clinical trials [7]. With increased R&D investment in the DMD market, patient advocacy, and regulatory incentives for drug development for rare diseases like DMD, we have seen advancements in gene therapy, extended use of steroids, and other novel treatments surface [17]. As DMD therapies advance, we expect these treatments to act as key driving factors for the DMD market [7]. In particular, the U.S. is expected to be a leader in clinical trials and remain dominant in the DMD regional market [7]. As anticipated by Market Research, with improved diagnosis methods, R&D focus and pipelines, and increased disease awareness, the market is anticipate to grow from 3.5 to 11 [7]. billion USD from 2023 to 2033 (Figure 6) [18]. Also of interest, with the recent COVID-19 pandemic, there was a rise in the DMD incidence rate, as patients with DMD were considered a high-risk population as they were more prone to experience severe COVID-19 symptoms [7]. This led to a greater DMD treatment adoption rate to combat the COVID-19 complications for DMD patients, thus increasing the expected market growth which is anticipated to maintain this upward trend through 2029 [7].

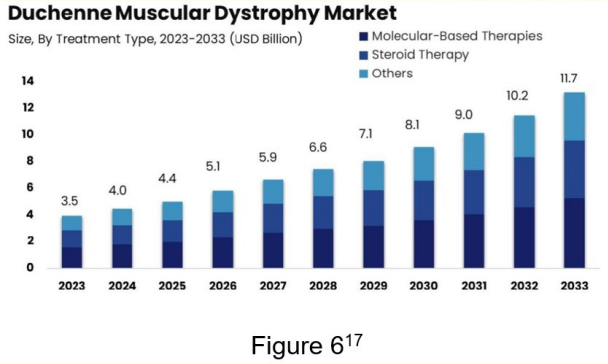


Figure 6[17]

Figure 6: Duchenne Muscluar Dystrophy Market [17].

The image shows the DMD market size and trends for molecular-based therapies, steroid therapy, and other treatments as reported by Market Research over 2023-2033.

Existing DMD products

Currently, there is no cure for DMD on the market, but several therapeutic options are available to help slow disease progression, stabilize symptoms, and treat associated conditions [19]. These therapies primarily include corticosteroids, disease-modifying treatments like exon skipping and gene therapy, supportive care such as physical therapy, respiratory support, cardiac care, and investigational therapies undergoing clinical trials (Table 1) [3,19-26]. Some leading companies in the DMD market are Sarepta Therapeutics, Pfizer Inc., PTC Therapeutics, FibroGen Inc., and F. Hoffman-La Roche AG [7]. Glucocorticoid therapy remains the gold standard for DMD patients due to its effectiveness in improving muscle strength and slowing the progression of muscle loss, inflammation, and other related symptoms [21]. The most commonly prescribed steroids for DMD are prednisone and deflazacort, primarily supplied by companies like EUROAPI, Coral Drugs Private Limited, Symbiotec Pharmalab, LGM Pharma, and Transo-Pharm USA LLC [22]. In 2023, the FDA approved vamorolone, a drug with a similar molecular structure as traditional corticosteroids created by ReveraGen BioPharma Inc [23]. Results from clinical trials showed vamorolone promoted improved muscle strength and stature without some of the side effects of the steroids such as stunted growth and weakened bones [23].

Treatment Approach	Examples	Major Contributing Companies	Advantages	Limitations
Glucocorticoids [19-22]	Prednisone, deflazacort, vamorolone	EUROAPI, Coral Drugs Private Limited, Symbiotec Pharmalab, LGM Pharma, Transo-Pharm USA LLC, ReveraGen BioPharma Inc.	-Improves muscle strength. -Decreases inflammation. -Delays cardiomyopathy. -Improves overall DMD patient survival rate.	-Long-term increases risk of adverse side effects like weight gain. -Does not cure the patient but works to relieve and delay symptoms.
Physical therapy [19,20]	Incorporate stretching, optimal positioning and splinting, orthotic standing devices, serial casting, and mobility devices.		-Acts to protect fragile muscles and preserve strength.	-Exercise may cause further muscle damage. -Does not address the underlying cause, only alleviates symptoms.
Respiratory therapy [19,20,24,25]	Annual pneumonia vaccine, lung volume recruitment, assisted coughing, nocturnal assisted ventilation, daytime ventilation, scoliosis surgery.	Pfizer, Merck Sharp and Dohme Corp., GE Healthcare, Medtronic, Fisher and Paykel Healthcare	-Relieves respiratory muscle fatigue, mucus plugging, atelectasis, pneumonia, respiratory failure, and potentially respiratory-induced cardiac arrhythmias.	-Hypoxemia may occur due to hypoventilation and ventilation-perfusion mismatch. -Does not address the underlying cause, only alleviates symptoms.

Cardiac management [19,20,26]	ACE inhibitors, beta blockers, ACE inhibitors, angiotensin receptor blockers (ARBs), noninvasive nocturnal ventilation, internal cardiac defibrillators, heart transplant	Pfizer, Novartis, AstraZeneca, Teva Pharmaceutical Industries, Johnson and Johnson Services, Merck KGaA	-Prevents ventricular cardiac remodeling. -Improves long-term cardiac outcomes.	-Does not treat the actual root issue, only works to relieve secondary symptoms. -Risk of adverse-drug side effects.
Gene transfer therapies [3,19,20,23]	SRP-9001, PF-06939926, SGT-001	Pfizer, Sarepta Therapeutics, Solid Biosciences Inc.	-Restores DAPC. -Decreases muscle fibrosis, kyphosis, and limb deformities.	-Challenging to get full length dystrophin expression.
Exon skipping agents [3,19,20]	Eteplirsen, golodirsen, casimersen, vitolarsen	Sarepta Therapeutics, Daiichi Sankyo Co	-Acts to restore and increase dystrophin levels. -Slows DMD progression. -Fewer cases of lost ambulatory function. -No serious adverse events reported.	-Not a cure, instead a delay of symptoms. -Risk of injection site reactions. -Some side effects like headaches and procedural pain.

Table 1: DMD Therapeutic Approaches [3,19-26].

A more novel drug type that has more recently made its way to the market for DMD is exon-skipping agents [7]. Exon-skipping is a very effective treatment strategies for restoring the expression of a functional dystrophin protein in DMD patients [7]. In some DMD patients, a deletion nonsense mutation in the dystrophin gene leads to premature termination of the transcript and improper dystrophin production [27]. As depicted in Figure 7, exon skipping restores the dystrophin protein by antisense oligonucleotides (AONs) binding to specific exons and inducing splicing in pre-mRNA [27]. The growing focus on exon-skipping technology has significantly propelled market growth [7]. For instance, eteplirsen (Exondys 51), produced by Sarepta Therapeutics, received fast-track approval from the FDA in 2016 for treating DMD patients with a mutation in the dystrophin gene amenable to exon 51 skipping [28]. In 2019, the FDA approved Sarepta Therapeutics' golodirsen (Vyondys 53) for DMD patients with a mutation amenable to exon 53 skipping [29]. In 2021, casimersen (AMONDYS 45), also manufactured by Sarepta Therapeutics, was approved by the FDA for DMD treatment targeting exon 45 skipping [30].

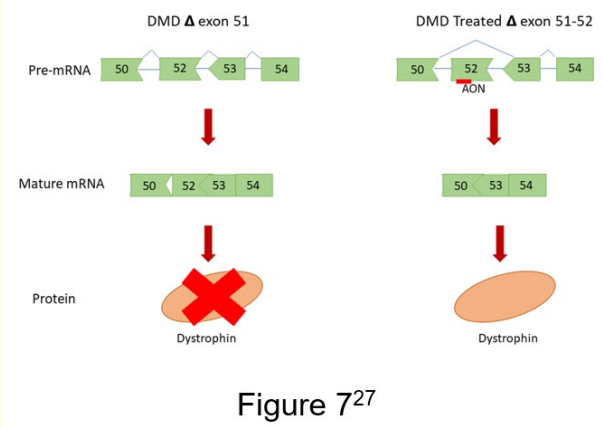


Figure 7: Illustration of the mechanism of exon skipping therapy by skipping of exon 52 [27].

The image shows the mechanism of exon skipping therapy of skipping exon 52. Exon skipping restores the dystrophin protein by antisense oligonucleotides (AONs) binding to specific exons and inducing splicing in pre-mRNA.

Clinical and preclinical trials

Clinical trials

In addition to the clinically applied treatment methods, many new emerging products are in the preclinical phase and may drastically expand the DMD market [3]. Some main therapies include *in vivo* gene correction, *in vivo* mRNA correction, upregulation of supporting molecules, enhancing muscle metabolism, novel steroids, and cell transplants [3]. This section investigates some of the most promising therapeutic options currently in clinical trials. The main products within clinical trials are listed in Table 2 [3,20,31-45].

In vivo gene correction aims to deliver the dystrophin gene to patients so their bodies can naturally synthesize the dystrophin protein [3]. Sarepta Therapeutics completed Phase III of a clinical trial for an open-label single-dose gene transfer therapy, known as delandistrogene moxeparvovec, which involves intravenous micro-dystrophin administration in boys with DMD [31]. The study, completed in 2023, found the injections to be well tolerated and resulted in long-term gene expression and phenotype improvements [3]. The drug has recently received FDA approval [3]. Additionally, Pfizer has been developing fordadistrogene movaparvovec, a gene therapy drug using an adeno-associated virus serotype-9 (AAV9) gene-replacement construct to introduce a dystrophin transgene into patients and restore functional dystrophin [32,33]. While Phase I clinical trials showed an acceptable safety profile and potential benefits for DMD patients by slowing muscle function loss, Phase III trials have been halted due to patient deaths [3,32].

In vivo mRNA corrections have also shown clinical benefits [3]. They utilize modified antibiotics to cause ribosomes to ignore stop codon sequences during the elongation transcription process, thereby overcoming nonsense mutations [3]. PTC Therapeutics, in their Phase III clinical trial of Ataluren, an orally delivered read-through *in vivo* mRNA correction drug, observed a reduction and delay in many DMD symptoms, including loss of ambulation and respiratory decline [3,34]. Ataluren is now approved by the European Medicine Agency (EMA) for treating male patients older than 2 years with DMD caused by a premature stop codon [35]. Exon skipping, another form of *in vivo* mRNA correction, involves drugs like eteplirsen, golodirsen, casimersen, and vitolarsen, all recently FDA-approved [20].

Another preclinical DMD therapeutic option being investigated is upregulating sarcolemmal supporting molecules, like utrophin, which have functions similar to dystrophin, such as forming dystrophin-glycoprotein complexes and assisting in muscle cell attachment to the basal lamina, respectively [3]. However, despite promising results in mouse models of DMD, these methods saw minimal efficacy in human trials [36]. A more novel supporting molecule, sarcospan, was found to alleviate muscular dystrophy in mouse models when upregulated, but sacrospan has not begun clinical trials [3].

Treatment	Treatment Type	Company	Clinical Trial Phase	Special Characteristics	Pros	Cons
Delandistrogene moxeparvovec (SRP-9001) [3,31]	*In vivo* gene transfer therapy	Sarepta Therapeutic	Phase III	Micro-dystrophin gene transfer using recombinant adeno-associated virus.	-Long-term dystrophin gene expression and phenotype improvements. -Well tolerated.	-Risk of off-site mutations. -Safe delivery not perfected.
Fordadistrogene movaparvovec (PF-06939926) [3,32,33]	*In vivo* gene transfer therapy	Pfizer	Phase III	Micro-dystrophin gene transfer using recombinant adeno-associated virus.	-Patients experienced dystrophin gene expression. -Improved North Star Ambulatory Assessment (NSAA).	-Vomiting and/or nausea seen in 40% of patients. -Phase III trial terminated due to patient death.

SGT-001 [3]	*In vivo* gene transfer therapy	Solid Biosciences Inc.	Phase II	rAAV9 delivery of micro-dystrophin.	-Phenotype improvement in 6-min walk test (6MWT) and NSAA scores.	-Variable dystrophin expression. -Severe adverse side effects including liver and kidney damage.
Ataluren [3,34,35]	Read through *in vivo* mRNA correction	PTC Therapeutic	Phase III	Induces readthrough nonsense mutation in the dystrophin mRNA.	-Reduces many of the disease symptoms, such as loss of ambulation and respiratory decline.	-Safe delivery not perfected.
Eteplirsen [3,20]	Exon-skipping *in vivo* mRNA correction	Sarepta Therapeutic	FDA approved	Exon 51 skipped.	-Average increase of 4% in dystrophin levels. -Dystrophin-positive fibers detected after 24 and 48 weeks of treatment. -Slower rate of decline in ambulation.	-Safe delivery not perfected. -Some drug side effects. -Requires continuous re-administration. -Mutation specific.
Vitolarsen [3,20]	Exon-skipping *in vivo* mRNA correction	NS Pharma	FDA approved	Exon 53 skipped	-All drug-treated patients experienced increased dystrophin levels. -Clinical benefits observed.	-Safe delivery not perfected. -Some drug side effects. -Requires continuous re-administration. -Mutation specific.
Golodirsen [3,20]	Exon-skipping *in vivo* mRNA correction	Sarepta Therapeutic	FDA approved	Exon 53 skipped	-Drug treated patients showed an average dystrophin increase of 16-fold. -Demonstrated clinical benefits.	-Safe delivery to all muscle cells is not yet perfected. -Some adverse drug reactions. -Must be continually re-administered. -Mutation specific
Casimersen [3,20]	Exon-skipping *in vivo* mRNA correction	Sarepta Therapeutic	FDA approved	Exon 45 skipped	-Demonstrated some clinical benefits.	-Minimal increase in dystrophin expression. -Safe delivery to all muscle cells not perfected. -Some adverse drug reactions. -Must be continually re-administered. -Mutation specific.

Utrophin [3,36]	Upregulation of supporting molecules	Summit Therapeutic	Phase II	Study stopped because of minimal efficacy.	-Low side-effects.	-Low efficacy.
Myoblasts [3,37,38]	Cell transplantation	CHU de Quebec-Universite Laval	Phase II	Donor myoblasts fuse with the patient's myofibers introducing the normal dystrophin gene.	-Dystrophin detected at injection site 4-weeks post-injection.	-Requires immune suppression. -Required high-density injections of cells ($3x10^7$ donor myoblasts)
Cardiosphere-derived cells [3,39]	Cell transplantation	Capricor Inc.	Phase II	Acts to treat cardiomyopathy secondary to DMD with allogeneic Cardiosphere-Derived Cells.	-No immune suppression required. -Reduced size of myocardial scars. -Additionally benefitted skeletal muscles.	-Only applies to patients with severely progressed DMD, limiting the number of patients that may benefit. -high-density injection
Human umbilical cord mesenchymal stem cells (UC-MSCs) [3,40]	Cell transplantation	Allergy and Asthma Consultants	Phase I	UCMSCs administered via IV and IM injection.	-No results posted	-No results posted
HLA-identical allogeneic mesoangioblasts [3,44]	Cell transplantation	FONDAZIONE CENTRO S. RAFFAELE DEL MONTE TABOR	Phase I/II	Systemic delivery of HLA-matched donor-derived mesoangioblasts (MABs) via intra-arterial transplantation.	-Systemic injection of cells. -Some dystrophin expression detection. -Relatively safe approach.	-Minimal fiber regeneration. -Minimal donor cell engraftment. -Minimal dystrophin expression -100s of millions of non-hematopoietic stem cells injected. -Low efficacy.
Autologous myoblast transplantation[3,41,42]	Cell transplantation	Assistance Publique - Hôpitaux de Paris	Phase I/IIa	Focus on OPMD patients via local intramuscular injections.	-Good efficacy. -Improved deterioration of swallowing function. -Partial repair of small muscle groups.	-Massive donor cell death upon transplantation.

Dystrophin Expressing Chimeric (DEC) cells [3,43]	Cell transplantation	Dystrogen Therapeutics Corp.	Phase II	Single intraosseous delivery of DEC cells to restore dystrophin expression without eliciting an immune response.	-Ambulatory patients showed improvements in 6MWT, NSAA. -Improved Performance of Upper Limb (PUL) test, strength and fatigue resistance, and Electromyography (EMG) parameters. -No immune suppression required. -Restores dystrophin expression in target organs.	-Dosing of 2×10^6 cells/kg. -Required matching of donor and recipient cells, so limited cell source.
Bone marrow-derived mesenchymal stem cells (BM-MSCs) and skeletal muscle-derived stem/progenitor cells (SM-SPCs) [45]	Co-cell Transplantation	National Science Centre grant and National Centre for Research and Development grant	Phase I	Co-transplantation of BM-MSCs and SM-SPCs directly into target muscle.	-Decreased creatine kinase levels. -Normalized profile of pro-inflammatory cytokines. -Improvement in motor unit parameters. -Dystrophin mRNA expression.	-High-density cell injections. -Required immunosuppression.

Table 2: DMD Treatment Products in Clinical Trial [3,20,31-45].

Cell transplantation is another promising approach under investigation, offering the potential to regenerate muscle tissue, reduce degeneration, and enhance muscle function [3]. In a Phase I/II clinical trial conducted by CHU de Quebec-Universite Laval, researchers explored the safety and effects on muscle strength of transplanting healthy parent donor myoblasts into the extensor carpi radialis, supplemented with immunosuppressants [37]. The study observed an increase in the percentage of myofibers expressing donor dystrophin, ranging from 3.5% to 26% four weeks post-injections [38]. Another study by Capricor Inc. investigated intracoronary infusion of cardiosphere-derived cells for 13 patients with substantial cardiomyopathy secondary to DMD [43]. In Phase II of the trial, patients received no immunosuppression, and results showed a significant reduction in scar size, improvement in inferior wall systolic thickening, and enhanced upper limb function [3,39]. Additionally, a Phase I/IIa clinical study examined autologous myoblast transplantation in patients with oculopharyngeal muscular dystrophy (OPMD), a rare genetic condition characterized by muscle weakness in the pharynx and upper eyelids, via local intramuscular injections [41,42]. Promising results were reported, with local administration demonstrating enhanced muscle repair efficacy and no deterioration in swallowing function observed over

two years, providing proof-of-concept for muscle cell therapy in muscular dystrophy [42]. Heydemann., *et al*. evaluated the safety and efficacy of Dystrophin Expressing Chimeric (DEC) cell therapy, where patient myoblasts were fused with healthy donor myoblasts for 3 DMD patients [43]. As illustrated in Figure 8, DEC cells, created through *ex vivo* fusion and delivered via systemic-intraosseous administration, interacted with the DMD patient's myoblasts, leading to improved muscle strength and function through dystrophin delivery [43]. The first-in-human study demonstrated promising safety and efficacy outcomes [43].

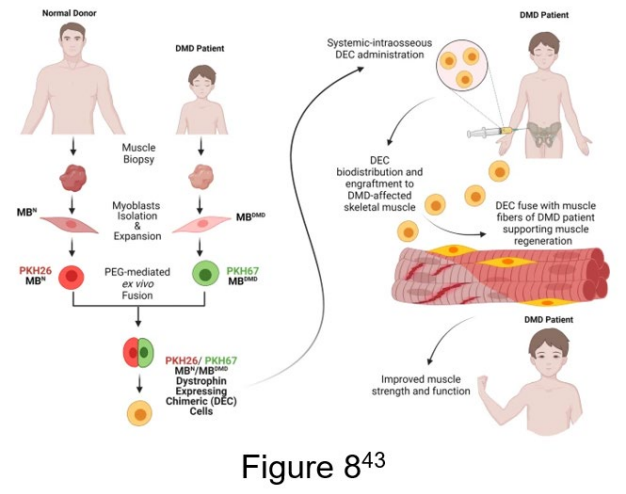


Figure 8[43]

Figure 8: Mechanism of action of the Dystrophin Expressing Chimeric Cell (DEC) cells created via ex vivo fusion of human myoblast from normal and DMD-affected donors. Followed by systemic-intraosseous administration causing DECs to engraft and fuse with the myoblasts of DMD patients. The DEC cells then deliver dystrophin and improve muscle strength and function [43].

This image shows how DEC cells, created through ex vivo fusion and delivered via systemic-intraosseous administration, interact with the DMD patient's myoblasts, leading to improved muscle strength and function through dystrophin delivery.

Preclinical Tissue Engineering Approaches for DMD Treatment

Cell therapy, while promising, has its challenges. The harsh environment and inflammation associated with DMD pose significant hurdles, leading to low cell viability and migration for transplanted cells [42]. These factors make it difficult to sustain the regeneration of the muscle tissue and achieve adequate improvement [42]. Therefore, increasing preclinical research has been investigating tissue engineering approaches for muscle injury and disease treatment [38]. Biomaterials and hydrogels offer a potential method to encapsulate and protect donor cells and growth factors during delivery for therapeutic repair [42]. They have the capability to transport cells or bioactive compounds for regenerating skeletal muscle tissue, either through transplanting muscle tissue fabricated *in vitro* or utilizing an injectable matrix that forms in the body, including pre-formed hydrogel particles (Figure 9) [42]. Table 3 displays the different tissue engineering designs, focusing on the two main approaches: pre-formed *in vitro* artificial skeletal muscle tissue and *in vivo* injectable delivery systems.

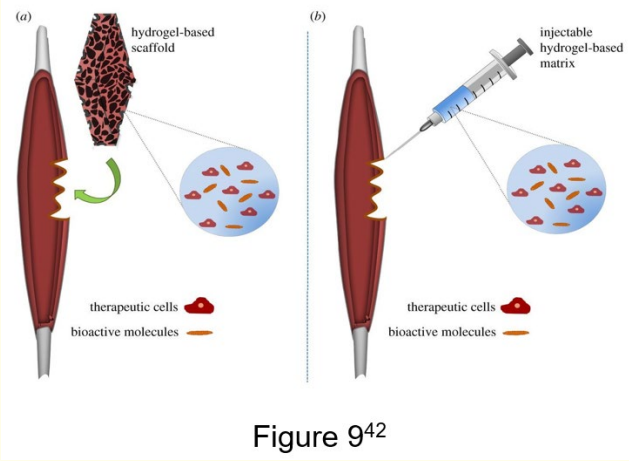


Figure 9[42]

Figure 9: Typical administration of hydrogel-based delivery systems for therapeutic cells and/or bioactive molecules in treatment of DMD [42].

a) Implantation of a pre-formed hydrogel-based scaffold. b) Injectable approach hydrogel-based matrix, which is a liquid precursor of the matrix that is injected and then formed in situ, or pre-formed hydrogel particles.

Tissue Engineering Approach	Research Group	Scaffold Biomaterial	Cell Type and/or Bioactive Molecule	Biofabrication technique	Pros	Cons
Pre-formed *in vitro* artificial skeletal muscle tissue	Nakayama., *et al*. [46-47]	Collagen	C2C12 myoblasts and human microvascular endothelial cells	Extrusion technique for spatially aligned pattern.	-Enhanced microvascularization, promoting improved blood flow. -Produced aligned and endothelialized muscle tissue leading to longer myotubes, synchronized contractility, and increased secretion of angiogenic cytokines.	-Results have not been investigated in the clinic.
	Yeo and Kim [46,48]	Alginate—PEO bioink with PLC	C2C12 myoblasts and HUVEC endothelial cells	3D printing and electrospinning.	-Biophysical and biochemical signals aid in aligning myoblasts. -Cell viability exceeded 90%.	-Risk of cell death due to high voltage during electrospinning process. -Alginate fibers easily degrade.
	Lee., *et al*. [46,49]	PLLA nonwoven scaffold with decellularized extracellular matrix (dECM) solution	C2C12 myoblasts and IGF-1	Thermal gelation.	-Enhances muscle regeneration upon transplantation. -Cell viability surpasses 90%. -Increased cell proliferation. -Promoted muscle tissue regeneration in a rabbit muscle defect model.	-Invasive surgery required for implantation of the scaffold. -Requires source for dECM, which risks immune response in recipients.
	Denes., *et al*. [46,50]	Gelatin	C2C12 myoblasts	Micropatterning	-Increased expression of sarcomeric genes observed. -Enhanced capacity for forming aligned sarcomeres. -Higher content of contractile proteins compared to myotubes cultured on unpatterned gelatin and plastic.	-Study only characterizes C2C12 myotubes *in vitro*; did not look at animal models.
	Borselli., *et al*. [46,51]	Alginate hydrogel	IGF-I/VEGF	Growth factors were encapsulated in alginate gel.	-Potential for controlled release. -Targeted delivery. -Improved skeletal muscle regeneration, revascularization, reinnervation, and functional recovery post-ischemic injuries. -95% recovery of injured ischemic muscle observed in mice.	-Risk of altering biological function of growth factors when covalently conjugated. -Rapid release of drugs due to use of physical entrapment.

	Falco., *et al*. [46,52]	PEG Scaffold	IGF-1/GFP encoding plasmid	Porous PEG scaffold made via leachable porogen strategy.	-Enhanced efficacy with potential for local and sustained release of therapeutic agents. -Demonstrated ability to deliver IGF-I and GFP genes to C2C12 cells *in vitro*. -Modifiable release of genetic substances via adjusting pore structure of scaffolds.	-Safety risks due to use of viral vectors.

Table 3: Preclinical tissue engineering designs with pre-formed *in vitro* artificial skeletal muscle tissue and *in vivo* injectable delivery systems) [46-59].

Much effort has been made to develop advanced scaffolds to mimic the skeletal muscle microenvironment and create artificial skeletal muscle tissue *in vitro* for reparative therapy [42]. Various approaches have been taken, with various scaffold biomaterial, cell types, and bioactive materials, to create a product that best assists with regenerating and improving muscle wasting diseases [42]. Nakayama., *et al*. synthesized a spatially aligned collagen scaffold embedded with C2C12 myoblasts and human microvascular endothelial cells [46,47]. When introduced into mouse models, researchers observed improved microvascularization, as well as the formation of endothelialized and aligned muscle tissue characterized by elongated myotubes, synchronized contractility, and increased secretion of angiogenic cytokines [46,47]. Lee., *et al*. also created a unique artificial skeletal muscle tissue using a poly(l-lactic acid) (PLLA) nonwoven scaffold synthesized with decellularized extracellular matrix (dECM) components and insulin growth factor-1 (IGF-1), embedded with C2C12 myoblasts (Figure 10) [46,49]. Other studies, such as Borselli., *et al*. and Falco., *et al*., have also investigated synthesizing scaffolds for delivering bioactive material, like growth factors or viral vectors, without cells [51,52].

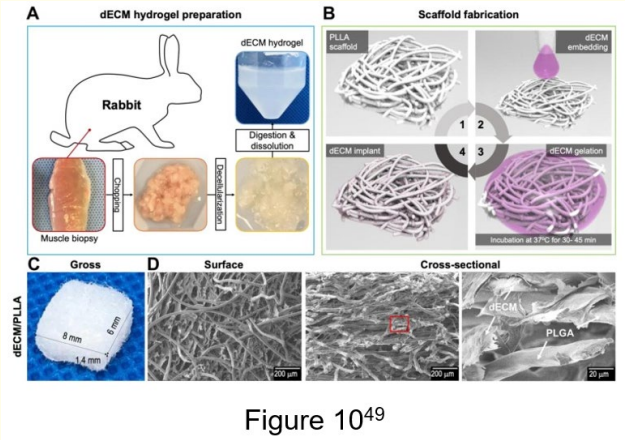


Figure 10: Illustration of dECM hydrogel preparation and scaffold fabrication [49].

(A) decellularization process using rabbit skeletal muscle biopsies and (B) PLLA scaffold fabrication. (C, D) Gross appearance, surface, and cross-sectional SEM images of the dECM/PLLA scaffold.

Additionally, biomaterials and hydrogels can be used as delivery vehicles for cells and bioactive molecules when treating skeletal muscle diseases [42]. As direct cell transplantation has seen challenges with cell viability and limited migration once implanted in the harsh *in vivo* environment, a delivery system may encapsulate and protect the cell and help promote integration processes with the host tissues [42]. For instance, Rossi., *et al*. developed an injectable photopolymerizable hyaluronan-based hydrogel to deliver MPCs in a controlled manner [53]. When this hydrogel-MPC complex was implanted into the ablated tibialis anterior

muscle of mice, it enhanced the capacity of myogenic precursor cells to regenerate muscle tissue and facilitate functional recovery [53]. Fuoco., *et al*. similarly utilized a PF hydrogel matrix to inject mesoangioblasts [54]. Other studies have also made injectable hydrogel beads or microspheres [55,56]. In particular, Kankala., *et al*. fabricated PLGA-based porous microspheres to deliver skeletal myoblasts (Figure 11) [56]. These microspheres provided a favorable microenvironment for the myoblasts to integrate with and deposit ECM, improving and facilitating cell adhesion, proliferation, and myogenic differentiation [56]. When investigated in the mouse model, improved cell retention and vascularization were shown, demonstrating cell-laden microcarriers' potential to promote muscle tissue regeneration through a minimally invasive delivery method [56]. Hwang., *et al*. and Ansari., *et al*. expanded on the cell delivery system and incorporated bioactive molecules, such as bFGF [58,59].

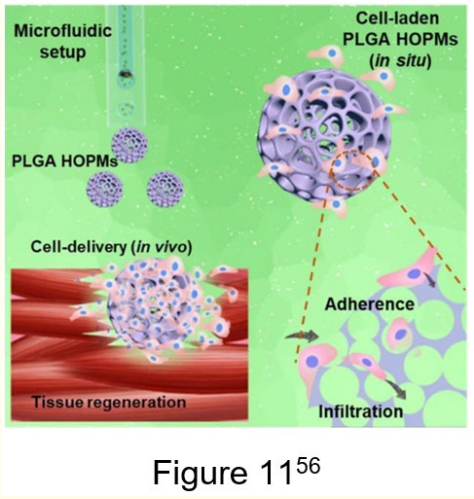


Figure 11[56]

Figure 11: Schematic of the PLGA HOPMs for minimally invasive cell delivery-based tissue regeneration [56].

This image displays Kankala., et al. fabricated PLGA-based porous microspheres to deliver skeletal myoblasts.

While there is increasing promise in tissue engineering methods for treating muscle tissue diseases and injuries like DMD, numerous challenges persist. Key among these are the methods of targeted implantation (i.e., localized injection versus surgical implantation), integration of scaffolds with host tissue, and the response of scaffolds to mechanical stimulation [38]. Addressing and optimizing these challenges are critical to progress toward developing enduring treatments that surpass current gold standards in the field.

Conclusion and Future Considerations

This article comprehensively reviews Duchenne Muscular Dystrophy (DMD), including an overview of the disease, its market size and trends, existing therapeutic options, products currently in clinical trials, and preclinical tissue engineering treatments. While there is increasing promise in tissue engineering methods for treating muscle tissue diseases and injuries like DMD, numerous challenges persist. Key among these are the methods of targeted implantation (i.e., localized injection versus surgical implantation), integration of scaffolds with host tissue, and the response of scaffolds to mechanical stimulation [42]. Addressing and optimizing these challenges are critical to progress toward developing enduring treatments that surpass current gold standards in the field. Future studies of tissue engineering DMD therapeutics should work to address significant limitations of current therapies, such as long-term expression of a functional dystrophin gene, restoring muscle regeneration capacity due to depleted muscle satellite cells, non-invasive delivery, and targeted delivery.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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