Nanoscale

REVIEW

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Cite this: DOI: 10.1039/d6nr01374k

Nanoparticle therapeutics in FSHD: current research and future perspectives

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Facioscapulohumeral muscular dystrophy (FSHD) is a hereditary neuromuscular disorder characterized by progressive, asymmetric muscle weakness caused by aberrant expression of the DUX4 (double homeobox 4) transcription factor. There are currently no disease-modifying treatments available, and treatment options remain restricted to supportive and symptomatic measures despite advances in understanding its molecular basis. Efforts have been made to develop therapeutic approaches targeting DUX4 silencing, genome editing, and downstream pathogenic pathway modification to address its unmet clinical need. Clinical translation is still hampered by the lack of effective, targeted delivery to skeletal muscle. Nanotechnology-based carriers are promising for overcoming these obstacles, as they improve tissue targeting while reducing off-target distribution and therapeutic payload. In this review, we address the current landscape of FSHD therapeutics and highlight how preclinical data on nanotherapeutics in Duchenne muscular dystrophy and other muscular dystrophies demonstrate the viability of nanoparticle-mediated strategies for muscle-targeted delivery and improved systemic bioavailability, making this an emerging approach in FSHD therapeutics. We also address issues with nanoparticle-based approaches for clinical use, including gaps in long-term safety, scalability, and efficiency. By integrating insights from the molecular genetics of FSHD and advances in nanomedicine in other muscular dystrophies, this review aims to provide a comprehensive perspective on the potential of nanotherapeutics and to outline future directions for their clinical translation in FSHD.

Received 7th April 2026,
Accepted 10th May 2026

DOI: 10.1039/d6nr01374k

rsc.li/nanoscale

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1 Introduction

Muscular dystrophies (MDs) comprise a heterogeneous group of rare, genetically inherited neuromuscular disorders primarily affecting skeletal muscle tissue. Globally, the estimated prevalence of MDs ranges between 19.8 and 25.1 cases per 100 000 live births. These conditions are marked by progressive muscle degeneration and impaired regenerative capacity. As the disease advances, it leads to significant functional decline, increased dependency, and reduced life expectancy.[1]

Based on different combinations of clinical, genetic, and pathological criteria, including the age of onset, progression rate, and pattern, along with the severity of symptoms and the distribution of muscle weaknesses, muscular dystrophies can

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be classified into nine major forms: myotonic, Duchenne, Becker, limb–girdle, facioscapulohumeral, congenital, oculopharyngeal, distal, and Emery–Dreifuss.[2,3] Among these, FSHD is the third most common inherited muscular dystrophy, with an estimated prevalence ranging from approximately 5 to 12 per 100 000 individuals, and is surpassed only by Duchenne and myotonic dystrophies.[4]

The disease was first described in the 19th century by French neurologists Landouzy and Dejerine, who characterized FSHD as a progressive, asymmetrical muscle weakness. This often begins in the facial muscles with the early involvement of the orbicularis oculi, which may result in patients sleeping with their eyes open. Further progression of the disease leads to weakness of the orbicularis oris, which impairs functions such as whistling and sucking. As the severity increases, shoulder girdle muscles such as the latissimus dorsi, serratus anterior, pectoralis major, subscapularis, rhomboids, trapezius, supraspinatus, infraspinatus, and deltoid become involved. This leads to increased difficulty in raising the arms above the head. Structural changes, such as horizontally positioned clavicles and scapular winging, are common. Some patients experience weakness in the pelvic muscles with selective distal involvement. In addition, patients may experience extramuscular manifestations, including sensorineural hearing loss, retinal vasculopathies, and, in some cases, cognitive impairments or epilepsy.[5]

The onset of symptoms in FSHD ranges from childhood to late adulthood, with more severe cases typically associated with an earlier onset. Based on the first appearance of the symptoms, the patients are typically categorized into three groups: infantile onset (<10 years), which is often severe and rapidly progressive; young-adult onset (15–30 years), which shows variable progression; and late-adult onset (52–74 years), which is typically milder. The severity and phenotypic diversity reflect the complex interplay between genetic architecture, epigenetic regulation, and modifier genes in shaping disease expression.[6–8]

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Genetically, FSHD is divided into two major subtypes: FSHD1 and FSHD2. FSHD1 is the most frequent form, accounting for about 95% of cases. It occurs due to the contraction of the D4Z4 macrosatellite repeat array on chromosome 4q35 to 1–10 repeats in patients. In contrast, healthy individuals typically have 11 to over 100 repeats of these units. Shortening of these repeats leads to partial relaxation and hypomethylation, thereby causing ectopic expression of double homeobox 4 (DUX4).[4] The DUX4 gene is a retrogene, located in the D4Z4 region (~3.3 kb) with restricted expression to germline cells and early embryonic stages, but it remains silenced in somatic tissues. However, unusual activation of DUX4 in somatic cells leads to FSHD disease pathogenesis as it induces cell death, oxidative stress, and inflammation, and affects myogenesis. To ensure stabilization and expression of its transcript, DUX4 requires a functional polyadenylation signal (PAS). This functional PAS is present in the 4qA allele, located downstream of the contracted D4Z4 array within a unique 260-base pair sequence known as pLAM, followed by a 6.2 kb β-satellite repeat. In contrast, the 4qB allele lacks this PAS and is therefore non-permissive for DUX4 expression, highlighting the importance of allele-specific variation in disease penetrance.[9]

FSHD2 accounts for about 5% of cases and is driven by a digenic epigenetic mechanism that disrupts the silencing of the DUX4 gene. Unlike FSHD1, patients with FSHD2 maintain a normal-length D4Z4 repeat array, usually consisting of at least 11 units on chromosomes 4q and 10q. However, due to heterozygous loss-of-function mutations in genes that regulate chromatin structure and DNA methylation, they show widespread hypomethylation in these regions. The most important of these are a few epigenetic modifiers, including structural maintenance of chromosomes flexible hinge domain containing 1 (SMCHD1), which mediates chromatin compaction and repression at the D4Z4 locus; DNA methyltransferase 3 beta (DNMT3B), which catalyzes new DNA methylation; and ligand-dependent nuclear receptor-interacting factor 1 (LRIF1), a chromatin-associated factor that interacts with SMCHD1 to reinforce transcriptional silencing. Mutations in any of these genes weaken the epigenetic integrity of the D4Z4 region, leading to chromatin relaxation and decreased methylation, which allows abnormal DUX4 expression in skeletal muscle. Similar to FSHD1, FSHD2 appears only when epigenetic suppression is associated with the permissive haplotype. Mutations in SMCHD1 have been shown to influence the clinical severity of FSHD1, especially in individuals with borderline repeat contractions, indicating a common epigenetic vulnerability across FSHD subtypes. Besides SMCHD1, whole-exome sequencing has identified additional candidates, including CTCF, DNMT1, DNMT3A, EZH2, and SUV39H1, that may further affect disease penetrance and variability when inherited alongside a permissive allele (Fig. 1).[10–12]

Historically, techniques such as Southern blotting, pulsed-field gel electrophoresis, and linear gel electrophoresis have been employed for the molecular diagnosis of FSHD1. However, in 2023, nanopore-based long-read sequencing combined with Cas9-targeted enrichment precisely resolved D4Z4

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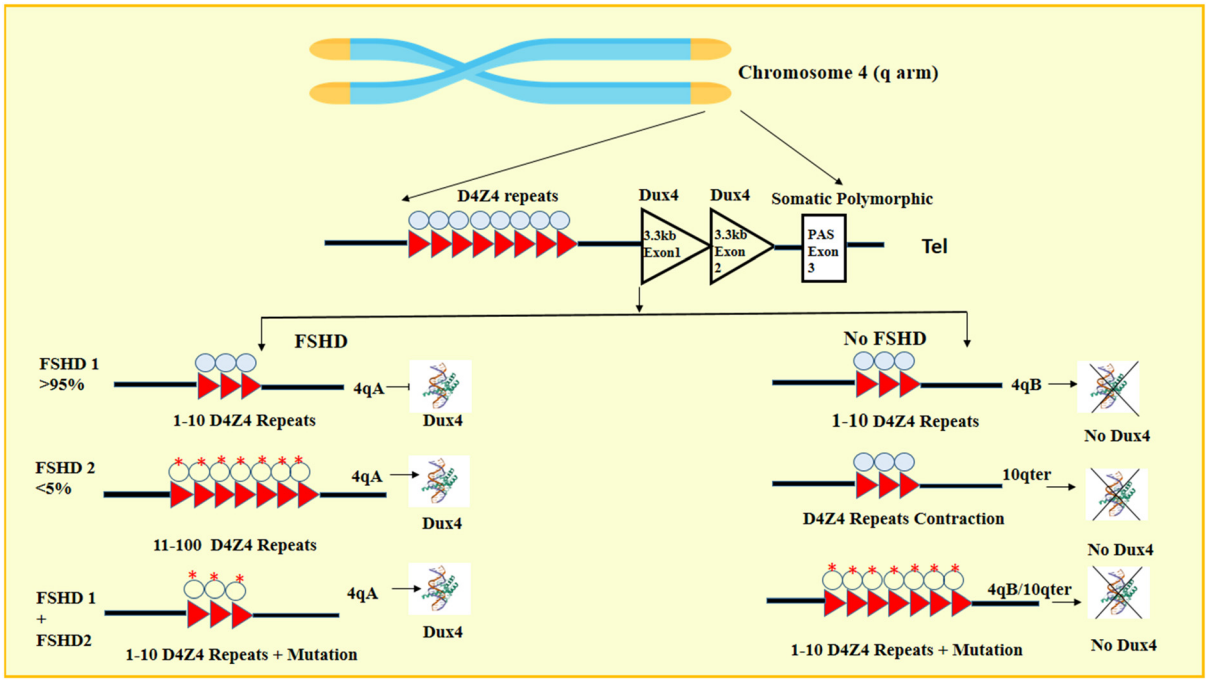

Fig. 1 The D4Z4 repeat array (red triangles) consists of 3.3 kb repeat units on chromosome 4 at the telomeric end. This array contains the exons of the DUX4 gene. Exon 3 of the 4qA allele contains a polyadenylation signal (PAS), which enables stable DUX4 expression in muscle and makes it disease-permissive. FSHD1 arises when the D4Z4 array is contracted to fewer than 11 units on a 4qA allele with normal chromatin modifier genes (light blue circles). FSHD2 is caused by the mutations in chromatin modifier genes (light blue hollow circles with red asterisks) (*e.g., SMCHD1, DNMT3B,* and *LRIF1*), again on a 4qA background. FSHD1 + 2 (combined form) involves both a moderately contracted D4Z4 array and mutations in modifier genes on the permissive allele. TEL = telomere.

repeat lengths, distinguished 4qA/4qB haplotypes, and quantified CpG methylation, offering a comprehensive, single-platform solution.[13] In addition, a 2024 clinical epigenetics study validated thresholds for methylation at the DUX4-PAS regions in 218 patients, achieving high diagnostic accuracy and demonstrating its usefulness in identifying at-risk, asymptomatic individuals.[14] On the other hand, FSHD2 can be easily diagnosed by utilizing both traditional sequencing techniques and modern platforms, such as next-generation sequencing and exome sequencing. These advancements have simplified testing for repeat size, methylation, and genetic changes, leading to more precise disease prediction.

Despite these advancements, FSHD currently lacks approved disease-modifying therapies,[8] mainly due to off-target effects and limited bioavailability of RNA-based drugs, gene-editing tools, and epigenetic modulators in muscle tissues.[15] Recent developments in nanomedicine, however, have improved its therapeutic options to address these drawbacks. For example, several of these nanocarrier platforms have already been tested in other forms of muscular dystrophy. These studies provide valuable proof of principle that nanoparticle systems can overcome the unique delivery barriers presented by skeletal muscle in MDs, including FSHD.

This review provides a thorough overview of current therapeutics in FSHD, focusing on the potential and applications of nanoparticles for treating other MDs by delivering gene-silencing agents and epigenetic modulators. Finally, we highlight multidisciplinary approaches integrating advances in nanotechnology, molecular biology, and clinical research to shape the future therapeutic landscape in FSHD, along with the research gaps that need to be addressed.

2 Therapeutic frontiers in facioscapulohumeral muscular dystrophy

Despite extensive research into the molecular biology of FSHD, it remains mainly focused on symptom management and supportive interventions.[16] Strategies such as personalized physiotherapy programs aimed at preserving muscle function, orthopedic interventions like scapular fixation to improve shoulder mobility, and assistive devices, including walkers and wheelchairs for the maintenance of patient autonomy, have been used. While these modalities certainly improve quality of life, they are unable to change the course of the disease.[17,18] Over the past few decades, pharmacological interventions have mostly failed to produce consistent or clinically significant improvements. Albuterol-β2-adrenergic agonists initially showed some potential in increasing muscle mass and selective strength measures. However, further trials revealed minimal benefits in overall function and high-

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lighted notable side effects, including tremors and sleep disturbances.[19,20] Likewise, corticosteroids such as prednisone, widely used in other muscular dystrophies, showed no significant improvement in muscle strength in FSHD.[21]

Losmapimod, a p38α/β MAPK inhibitor developed by Fulcrum Therapeutics, generated significant interest early on because it was one of the first attempts to directly interfere with the molecular pathway that drives FSHD. The idea was to reduce p38 activity and, in turn, limit DUX4 expression. Initial work, including the ReDUX4 Phase 2 study, demonstrated changes in some disease biomarkers and a potential slowing of muscle decline. These findings encouraged the company to proceed with the Phase 3 REACH trial (NCT04003974), a fairly large, international study that enrolled more than 260 patients with confirmed FSHD1 or FSHD2. The trial primarily focused on upper-limb function, while also tracking strength, MRI changes in fat fraction, and patient-reported measures. The results, however, were not what many hoped for. Even though the drug clearly hit its intended target, the trial did not show any meaningful difference between the losmapimod and placebo groups in the primary or secondary outcomes. This was a setback, but it pointed out gaps in current trial designs, especially regarding patient selection, endpoint sensitivity, and how long patients need to be followed up to see a measurable change. Although Fulcrum eventually halted the development of losmapimod for FSHD, the experience has been valuable for further research and helped refine the strategies that newer therapeutic programs are now using.[22]

A game-changing approach in FSHD therapy lies in directly targeting the DUX4 transcript or its protein product. Preclinical research using oligonucleotides, such as gapmers, antisense oligonucleotides (ASOs), and small interfering RNAs (siRNAs), has shown significant decreases in downstream toxicity and DUX4 mRNA levels in animal models and patient-derived myotubes.[16] However, challenges such as systemic delivery, tissue-specific uptake, and ensuring sustained bioavailability in skeletal muscle still persist. Recent innovations have tried to address these bottlenecks. Avidity Biosciences developed AOC 1020, an antibody–oligonucleotide conjugate that couples DUX4-targeted siRNA to a monoclonal antibody that binds the transferrin receptor (TfR1), hence facilitating targeted delivery into muscle tissues. Interim results from the Phase 1/2 FORTITUDE trial of delpacibart braxlosiran (del-brax) reported a robust reduction of more than 50% in DUX4-regulated gene expression, along with encouraging safety and tolerability profiles. This success resulted in the launch of the global Phase 3 FORWARD trial, with the potential to establish AOC 1020 as a first-in-class DUX4-silencing therapy[23,24] with 12 + additional trials planned. A similar approach was taken by Dyne Therapeutics, wherein they demonstrated the potential of DYNE-302 for achieving functional improvement in FSHD. DYNE-302 is a DUX4-siRNA conjugated to anti-human TfR1. Preclinical data have shown that DYNE-302 exhibits strong gene-silencing potency in *in vitro* and *in vivo* studies; however, conclusive clinical trial data are awaited.[25]

The precision offered by genome-editing technologies like CRISPR/Cas9 also opens the door to potentially curative interventions for FSHD. CRISPR–Cas9 is a versatile genome-editing tool for targeted gene modification and therapeutic applications.[26] It has been actively pursued in preclinical models of FSHD for the deletion of the polyadenylation signal necessary for DUX4 mRNA stability or editing epigenetic regulators such as SMCHD1 to restore chromatin repression at the D4Z4 locus. As a proof of concept in 2021, epigenome editing using dCas9-KRAB was demonstrated, wherein Cas9 was repurposed as the dead Cas9 (dCas9) and fused to the Krüppel-associated box (KRAB) domain, a potent transcriptional repressor that can be fused to heterologous DNA-binding protein repressor constructs to repress long-term DUX4 expression in FSHD muscle cells, offering a durable silencing approach. In the study, primary FSHD cells were transfected with lentiviral vectors carrying dCas9-KRAB and specific sgRNAs designed for targeting distinct regions within the D4Z4 repeat, as well as sequences flanking it, such as DUX4 exon 3. The results showed that directing dCas9-KRAB to the DUX4 promoter or exon 1 led to decreases in DUX4 expression and its downstream target genes by up to 45% and 60% of their normal endogenous levels, respectively. However, the safety, efficiency, and ethical complexities of germline or somatic gene editing necessitate cautious clinical translation.[27]

Independent of DUX4 targeting, myostatin, a potent negative regulator of muscle growth, has emerged as a therapeutic target in FSHD. MYO-029, an early-generation myostatin-neutralizing antibody, was studied, though it failed to produce significant gains in muscle function during early trials, possibly due to sub-therapeutic tissue concentrations.[28,29] To tackle this, more refined agents, such as GYM329 (also known as REGN1033), are under investigation in the ongoing MANEUVRE trial (NCT05548556), focusing on genetically confirmed FSHD1/2 cohorts. This newer candidate exhibits improved binding affinity and a prolonged half-life, and initial safety data are promising, with efficacy readouts expected soon.[30,31] In addition to these, muscle-building strategies, including dietary supplements, are also in focus. Creatine, a guanidino compound, may improve muscle strength and increase fat-free mass due to its antioxidant properties, with the ability to reduce protein degradation.[32,33] It has been tested in an open-label trial involving 29 participants, in which a loading dose of 20 g per day was administered for one week, followed by 5 g per day for eight weeks and then a maintenance dose of 5 g per day for eight weeks. Several assessments were conducted before and after the intervention; although the results were satisfactory in terms of muscle strength, 10 individuals reported adverse effects during the study.[32] Thus, concrete data analysis is needed to support the treatment of FSHD and pave the way for its market approval. Additionally, Dr Dalila Laoudj-Chenivesse and team led clinical studies on antioxidant supplementation with vitamin C, zinc, and selenium, as these deficiencies are linked with oxidative stress, metabolic dysfunction, and mitochondrial anomalies in FSHD.[34–36] These trials have shown measurable reductions in systemic oxidative stress markers and modest functional improvements in FSHD patients. However, it has been

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suggested that the absence of tightly controlled dietary intake in these studies necessitates careful interpretation of the data.[37] Novel metabolic interventions such as NAD + boosters, PGC-1α activators, and mitochondria-targeted peptides are also under investigation, with the aim of rejuvenating bioenergetics and reducing DUX4-induced cellular stress.[38–40]

Besides nutritional supplements, protein-based therapeutics such as Resolaris (ATYR1940) have been evaluated for their potential to improve muscle function in FSHD. Developed by aTyr Pharma, Resolaris is a first-in-class intravenous recombinant protein drug derived from naturally occurring aminoacyl-tRNA synthetase enzymes traditionally known for their role in protein synthesis and immune-modulating properties. This therapy aimed to target inflammation in FSHD and it showed promising improvements in manual muscle testing and quality-of-life. However, no peer-reviewed results from the Phase 1b/2 placebo-controlled and follow-up trials (NCT02239224, NCT02603562, NCT02579239, NCT02836418) are yet available (Fig. 2).[41]

All these trials hint that successful FSHD clinical trials should involve outcome-measurement strategies as the disease onset and severity vary widely.[42] Therefore, outcome assessments in studies and clinical trials are typically categorized into three main groups: clinical outcomes, which aim at monitoring the patient's functional status and disease progression. There are patient-reported outcomes, that solicit patients' own input through questionnaires, such as the FSHD Health Index and the FSHD Rasch-built Overall Disability Scale, to capture their personal experience of the disease. The third and most important point is the measurement of biomarkers, indicating disease presence or progression, evaluating treatment response, or monitoring safety. Integration of advanced clinical outcome assessments, such as the FSHD Composite Functional Index and digital biomarkers derived from wearable devices, is enhancing the sensitivity of clinical trials and enabling personalized monitoring of disease progression and therapeutic response.[43]

In summary, the landscape of FSHD therapeutics is shifting from symptomatic support to mechanism-driven, precision interventions. The growing pipeline of targeted therapeutics, coupled with an improved understanding of disease heterogeneity, now sets the stage for combination therapy designed to concurrently suppress DUX4, boost muscle mass, and correct metabolic imbalances that may ultimately alter the natural history of FSHD.

3 Nanoparticle platforms in muscular dystrophy therapeutics: redefining precision medicine in the 21st century

Nanoparticles (NPs) are particles of sizes ranging from 1 to 200 nanometers. Due to their modifiable physicochemical properties and targeted drug-delivery capabilities, NPs have significantly revolutionized disease therapeutics across a variety of medical domains. As of now, over 60 nanomedicine formulations have been approved for clinical use, encompassing applications in oncology (*e.g.*, Doxil and Abraxane), iron-deficiency therapies (*e.g.*, Ferumoxytol), antifungal treatments (*e.g.*, AmBisome), macular degeneration, anesthetics, and even rare genetic disorders.[44,45]

Because of their shape, size and charges on the surface, nanoparticles can efficiently enter cells through various mechanisms.[46] Once internalized, they facilitate the controlled intracellular release of therapeutic agents, thereby allowing modulation of signaling pathways and gene expression to correct

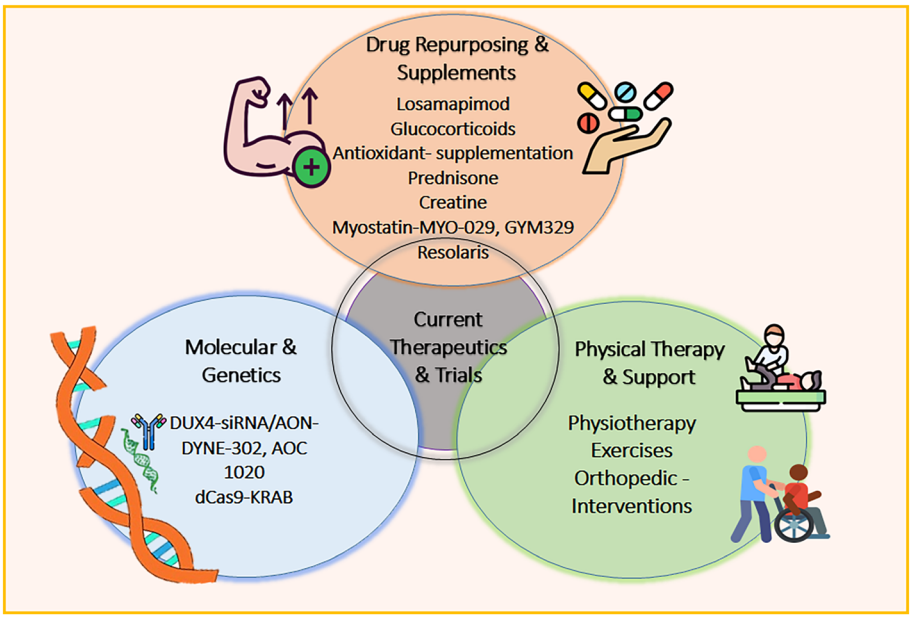

Fig. 2 Summary of the current therapeutics and trials landscape in FSHD.

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disease.[47] Recent research has highlighted both passive and active targeting strategies using nanoparticles, showing improvements in therapeutic indices,[48] as well as stimuli-responsive nanoparticles that can respond to specific triggers such as pH changes, enzyme activity, reactive oxygen species (ROS), or temperature.[49] Based on their structural composition, nanoparticles are broadly categorized into inorganic and organic systems, each with unique advantages in biomedical applications. The detailed categorization and properties of these nanoparticles have been thoroughly reviewed in the literature.[50–53]

In muscular dystrophies, including FSHD, therapeutic strategies primarily involve glucocorticoids (GCs), physical therapy, and emerging gene-based interventions. However, chronic administration of GCs has significant adverse effects, and gene editing and gene delivery need a carrier that can enhance blood circulation kinetics, protect the payloads, and maintain specificity.[54] The progress in nanotherapeutics has underscored the potential of nanocarriers to deliver drugs, genes, and gene-editing tools while preserving their efficacy with minimal exposure. This section covers the advancements in nanotherapeutics for muscular dystrophies (Fig. 3).

3.1 Nanoparticle-mediated drug delivery and drug repurposing

Drug repurposing accelerates clinical application and reduces costs by leveraging existing knowledge of a drug's safety, pharmacokinetics, and mechanism of action. It also makes it possible to find efficient treatments for complicated illnesses like MDs more quickly. Nanocarrier technology and drug repurposing can be combined to maximize therapeutic efficacy, targeted localization, and controlled release and reduce systemic side effects.[55] For instance, rapamycin, an immunosuppressant and mTOR (mammalian target of rapamycin) inhibitor, is known to enhance autophagy and thereby limit muscle damage. However, rapamycin alone, even at 4 times the clinically recommended oral dose, did not significantly improve skeletal muscle strength in mdx mice. These limitations are largely attributed to poor tissue targeting and dose-dependent toxicity, which limit its clinical translation in MDs. Perfluorocarbon (PFC)-based nanoparticle delivery systems have been tested to address these limitations. PFC-NP, due to their chemical and biological inertness, reduced the likelihood of adverse effects from long-term dosing. Intravenously administered rapamycin NPs significantly increased grip strength and improved cardiac contractility, demonstrating superior bioavailability and muscle reach after only eight treatments.[56]

Another example is Pentamidine (PTM), an FDA-approved antiparasitic drug, which has been repurposed to treat splicing defects in myotonic dystrophy type 1 (DM1) though its toxicity limits its clinical use. However, PTM encapsulated in biocompatible and biodegradable materials, such as hyaluronic acid (HA) and poly(L-arginine) derived nanoparticles, showed minimal toxicity in *in vitro* studies conducted on C2C12 cells.

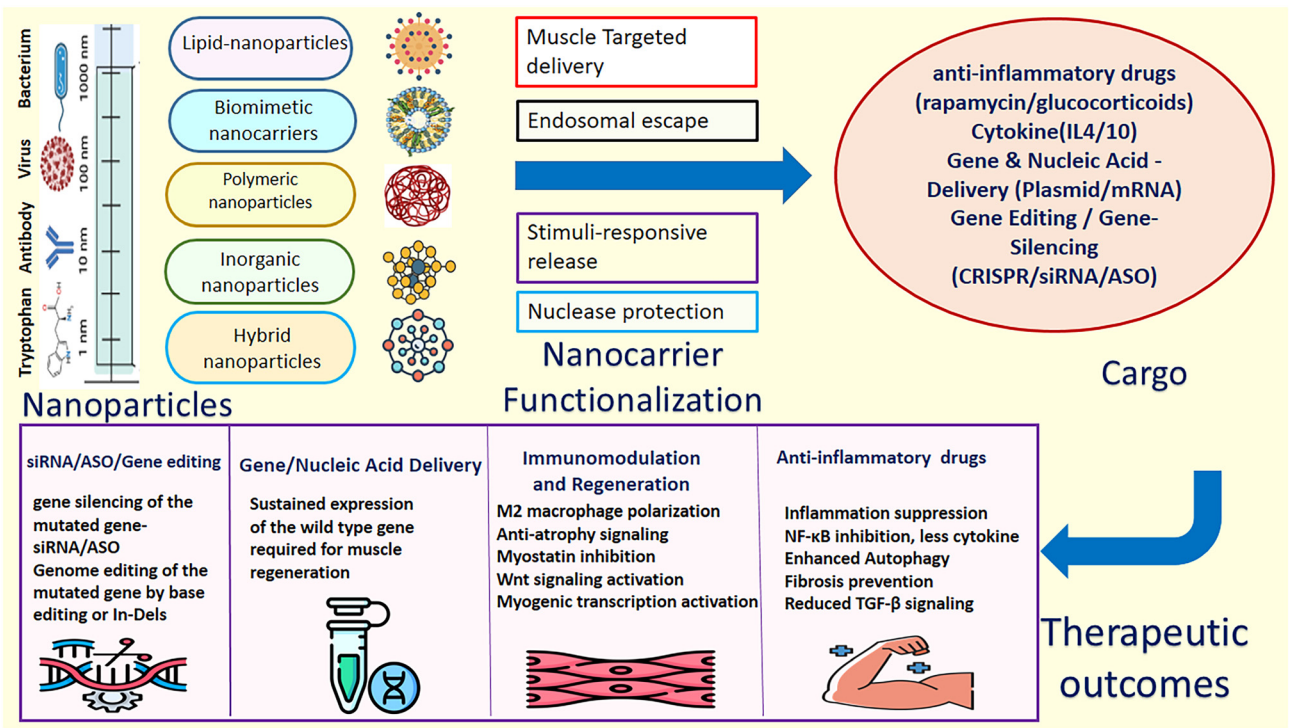

Fig. 3 Mechanistic overview of nanotherapeutic strategies in muscular dystrophies. This summarizes nanoparticle-based encapsulation of diverse cargos, their delivery to skeletal muscles *via* passive and active targeting mechanisms, as well as their cellular uptake, intracellular release, and modulation of key molecular pathways involved in muscle degeneration, inflammation, and regeneration.

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HA, a key component of the extracellular matrix, plays an important role in muscle repair and regeneration by interacting with CD44 receptors widely present on cell surfaces. This study showed endosomal escape using hyaluronic acid-based nanocarriers.[57] Furthermore, *ex vivo* experiments confirmed effective uptake of nanoparticles by skeletal muscle fibers, and an additional reduction in nuclear foci in a novel DM1 cell model.

Superparamagnetic iron oxide nanoparticles (SPIONs) are being used in various biomedical applications due to their biocompatibility and unique superparamagnetic properties. L-Cysteine-functionalized SPIONs have been used as targeted carriers for deflazacort and ibuprofen, two immunosuppressants in DMD, with 8 nm cores (120 nm hydrodynamic diameter). These NPs accumulated effectively in dystrophic muscle under magnetic guidance, reducing systemic exposure and preserving liver and kidney function. Normal creatine kinase levels in blood indicated reduced muscle damage. These SPIONs combine targeting, imaging, and therapy, serving as theranostic platforms.[58] Similarly, in a recent research study, gentamicin has been repurposed and delivered by hybrid liposomes composed of L-α-dimyristoylphosphatidylcholine and polyoxyethylene lauryl ether nanoparticles. Gentamicin, an aminoglycoside, can suppress stop codons in the dystrophin gene, restoring partial dystrophin expression *in vitro* and *in vivo*. These sterically stabilized nano-liposomes enhance dystrophin expression while minimizing toxicity and systemic side effects and exploit the abnormal blood vessels in inflamed tissues. This allows them to pass through the leaky vasculature and become trapped by inflammatory cells, thereby enabling passive targeting and accumulation within the affected areas.[59]

PEGylated (polyethylene glycol – PEG)) nanoparticles have been widely used to enhance circulation time, reduce immunogenicity, and improve biodistribution. Research with PEGylated liposomal methylprednisolone hemisuccinate, used for intravenous treatment in mdx mice (~80 nm diameter), preferentially lodges in the inflamed diaphragm, the most severely affected muscle in the early course of the disease. After 4 weeks, these steroidal nanodrugs reduced TGF-β, macrophage infiltration, and fibrosis. Long-term (58 weeks) treatment of mice showed notably improved muscle strength and mobility compared to the control group injected with the same steroid.[60] However, PEGylation also presents challenges, such as the potential for "accelerated blood clearance" upon repeated administration and possible interference with cellular uptake, which may limit its long-term therapeutic effectiveness.[61]

3.2 Nanoparticles for immunomodulation and regeneration

In muscular dystrophy, immune inflammation is very common, which results from the infiltration of inflammatory cells, such as T lymphocytes. These inflammatory responses play a significant role in the development of muscle fibrosis. Hence, a more effective treatment could be a combination therapy with anti-inflammatory cytokines and myogenic factors. Beyond drug delivery, nanoparticles are increasingly applied in immunomodulation and muscle regeneration.

In a groundbreaking study called PA4 treatment, gold NPs (AuNPs) were conjugated with IL-4 (and potentially IL-10), achieving a fourfold rise in muscle contraction force and velocity after two weeks in mdx mice. Cytokines such as IL-4 and IL-10 are well known for promoting immune responses and muscle repair by stimulating M2 macrophages and facilitating muscle regeneration. Nonetheless, these cytokines require localized delivery to achieve benefits while avoiding side effects. Injectable formulations, like AuNPs, with the ability to deliver treatments specifically to muscles while minimizing toxicity and immune reactions in humans, offer a promising approach.[62] The strategy leverages immunomodulation to improve muscle function, demonstrating how NPs can precisely deliver cytokines.

Among various NPs, dendrimers have emerged as promising nanostructures in pharmaceutical applications. These highly branched, surface-modifiable polymers are nanoscale in size, highly water-soluble, and biocompatible, and have well-defined molecular weights. Their internal cavities and polyvalent surfaces make them ideal carriers for safe and efficient drug delivery.[63] In one of the studies, poly(amidoamine)-hydroxyl-terminated dendrimer (PAMAM-OH) was used to protect angiotensin(1–7), forming stable complexes with about 50–65% peptide coverage. Ang-(1–7) has anti-atrophic effects, and this property of Ang-(1–7) underscores its therapeutic potential. However, Ang-(1–7)'s instability limits its effectiveness when administered orally or by injection. Ang-(1–7)-loaded PAMAM dendrimers restored muscle fiber size and contractile strength in atrophy models more effectively than the free peptide. Similarly, nanolipodendrosomes co-loaded with glatiramer acetate (an anti-inflammatory cytokine upregulator) and MYOD (a key myogenic transcription factor) reduced inflammatory markers (CD4+/CD8+) and increased muscle mass, highlighting the potential of combinatorial nanotherapy.[64]

In addition to inflammation, muscle atrophy and fat infiltration (replacement of muscle tissue with adipose tissue – myosteatosis) are two significant attributes of the progressive muscle weakness in muscular dystrophies. WNT7A (Wingless-Type MMTV Integration Site Family Member 7A) is a protein with a role in muscle growth and the reduction of fat accumulation in muscles. In a 2025 study, researchers tested the efficacy of WNT7A-loaded lipid nanoparticles *in vitro* and *in vivo*. Experiments were done on primary murine fibro-adipogenic progenitors, C2C12 myoblasts, and mouse models with glycerol-induced muscle injuries. W7a-LNP treatment increased muscle fiber size and decreased fat buildup in muscles. These results indicated that this mRNA LNP complex is a promising alternative to protein therapies for treating muscle degeneration.[65]

Similarly, successful delivery of follistatin messenger RNA (mRNA) by using polymeric nanoparticles demonstrated growth by inhibiting negative regulators of muscle growth like myostatin and activin A. When administered subcutaneously,

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it increased lean mass in mice after eight weeks of repeated injections compared to untreated controls.[66] However, further research is required for optimization of dosing, delivery methods, and distribution for clinical translation.

3.3 Nanoparticles for gene and nucleic acid delivery

Alongside protein and cytokine delivery, the revolution in nanoparticle-based gene and nucleic acid delivery is also a notable advancement in treating MDs.[67] They present a viable alternative to conventional AAV viral vector-based therapy, which poses risks of strong adaptive immune responses, as well as poor packaging and tissue specificity.[68] Therefore, researchers are increasingly exploring the potential of NPs for gene delivery. Recently, R-HAp NPs (~calcium phosphate core, surface-modified with arginine) have been used to deliver full-length dystrophin plasmids (~18.8 kb) to primary mouse and human myocytes and patient-derived cells. Notably, effective expression was achieved with only 50 ng of plasmid, 20× less than Lipofectamine 3000, with sustained mRNA/protein expression for up to a week.[69] This non-viral, cost-effective strategy overcomes AAV size limitations and viral immunogenicity; however, *in vivo* studies are yet to be done to show its efficacy in an animal model.

Similarly, hyperbranched poly(ester amine) (PEA)-based NPs exhibited strong intracellular trafficking and serum stability when delivering plasmid DNA to mdx mice and muscle cell types (C2C12 myoblasts, human skeletal cells). They resist serum-induced dissociation and DNase degradation, enabling lower doses and sustained expression with minimal cytotoxicity.[67] Furthermore, in an immuno-compromised mdx model, lipid-coated mesoporous silica nanoparticles displayed high biocompatibility, low off-target accumulation, and potential for payload delivery combined with imaging modalities.[70] However, when tested at different concentrations with long-term incubation times, these nanoparticles exhibited toxicity in primary human myoblasts compared to polymeric nanoparticles.[71]

3.4 Nanoparticles for gene modulations and gene editing in MDs

Alternatively, muscle disorders can be treated by suppressing the mutated gene by using ASOs, siRNAs, and miRNAs (microRNAs). However, their poor cellular uptake, instability, and rapid renal clearance pose a concern.[63] Nanoparticles have been studied for their ability to deliver these compounds to target exon-skipping or gene silencing in MDs.[72,73]

Early studies using polymeric AON (antisense oligonucleotides) NPs and PMMA (poly(methyl methacrylate)) NPs restored dystrophin in mdx mice, achieving results unmatched by naked AONs.[74] In recent approaches, PEG-PEI-AO (polyethylene glycol and polyethyleneimine polyplexes) embedded within PLGA (poly(lactic-*co*-glycolic acid)) nanospheres achieved 60–100% encapsulation, shielding the cationic charge and sustained release (20% burst + 65% sustained release over 3 weeks), while attenuating surface charge-related toxicity.[75] Various nanoparticle-based strategies for the delivery of therapeutic oligonucleotides have been effective *in vitro* and *in vivo* for muscular dystrophy, but none have adequately addressed the specificity towards muscle satellite cells (MuSCs), which are crucial for muscle regeneration. Francesco *et al.* demonstrated that α7/β1 aptamer-conjugated AuNPs loaded with miR-206, a muscle-specific microRNA that enhances MuSC function, successfully targeted MuSCs and restored their regenerative capacity in dystrophic muscles of D2-mdx mice. The result was consistent with findings that α7/β1 AuNPs are non-cytotoxic.[76]

Even though nanocarriers can be suitable candidates for the delivery of ASO, siRNA, and miRNA, not all of them demonstrate efficient delivery. For example, perfluoropentane-based chitosan nanobubbles loaded with phosphorodiamidate morpholino oligomer (PMO)-AONs for silencing DUX4 expression in an FSHD cell model failed to downregulate DUX4 and its downstream genes. This highlights the irrevocable interaction between chitosan-shelled NBs and PMO-AONs.[77]

The potential of nanoparticles is not limited to gene therapy and oligonucleotide-based therapy; their ability as a bio-carrier in the context of gene editing for MDs is also being explored. For the delivery of CRISPR-Cas9, in DMD AAV, micro-dystrophin vectors have advanced to clinical approval, yet limitations persist: capsid size constraints, systemic distribution inefficiencies, immunogenicity, manufacturing inconsistencies, and high costs.[78] Another crucial challenge is the large size of Cas9 and its degradation in the body's serum.[79] Hence, efforts are being made to deliver Cas9/sgRNA ribonucleoprotein complexes using non-viral methods to enhance therapeutic outcomes. In one of the recent studies, CRISPR RNP-loaded, PEGDB (poly(ethylene glycol)-*b*-poly(*N*,*N*-dimethylaminomethacrylate-*stat*-*n*-butyl methacrylate))-coated PSiNP(porous silicon nanoparticles nanocomposites) with 20 nm pores allowed efficient loading. The silicon density and PEGDB coating, where PEG provided stability and DB helped the particles respond to pH release of the cargo effectively into cells, contributed to effective delivery irrespective of the protein's size or charge. *In vitro* studies demonstrated twice the gene-editing efficiency compared with commercial reagents such as Lipofectamine and CRISPRMAX. Local or intravenous administration of the nanocomposite in the *in vivo* DMD model suggested successful gene editing.[80]

Similarly, lipid nanoparticles have been tested for the delivery of Cas9-sgRNA to multiple tissues *via* IM injection in DMD mice, including the muscle, lungs, and liver. After three weeks of the final injection, western blotting and immunofluorescence assay showed successful restoration of the dystrophin gene.[81] In another research, guanidinium-rich lipopeptide-based nanoparticle delivery of CRISPR-Cas9/sgRNA ribonucleoprotein (RNP) restored dystrophin production, decreased skeletal muscle fibrosis, and markedly enhanced muscle strength in a DMD mouse model.[82] An AuNP-CRISPR complex coated with cationic endosomal-disrupting polymers successfully delivered CRISPR-Cas9 proteins and nucleic acid components, exhibiting low off-targeting and high efficiency in correcting DNA mutations in DMD mice upon local injection.[83]

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In DMD, mutations in the exons lead to low to no production of dystrophin protein, and one way to produce the functional dystrophin is by skipping the faulty parts of the exon, known as exon skipping. CRISPR-Cas9 technology has been leveraged for exon skipping in DMD to restore functional dystrophin. However, the requirement for a protospacer adjacent motif (PAM) for wild-type Cas9 to cut the DNA in the right spot limits its reach near the gene where skipping is required. To address this, researchers developed biomineralized nanoparticles encapsulating PAM-less Cas9 (SpRY) pDNA (Bm-SpRY NPs) for *in vitro* and *in vivo* DMD gene editing. These nanoparticles showed high encapsulation efficiency, excellent biocompatibility, protection against enzymatic degradation, and efficient delivery even under high-serum conditions. Testing multiple PAMs for the DMD exon 51 splice acceptor site revealed that the TAG PAM target region had the highest editing efficiency, with preferential mutations. *In vivo*, intramuscular injection of Bm-SpRY NPs achieved DMD gene editing in muscle tissue without detectable damage, demonstrating the potential of this approach to broaden CRISPR-based therapeutic applications for other MDs.[84]

Cas9 nucleases are most frequently used for generating DNA breaks, which can lead to unwanted insertions and deletions. However, recent advances have overcome this limitation through the introduction of base editing and prime editing. For example, Jiang *et al.* used lipid nanoparticles–adenine base editors in a Tyrosinemia I mouse model to correct the causative point mutations.[85] However, in the context of FSHD, research in this area remains largely unexplored, highlighting a promising strategy with significant therapeutic potential for correcting mutations in chromatin remodelers, such as SMCHD1 and DNMT3B, in FSHD2.

Collectively, these studies underscore the multifaceted potential of nanoparticles as vehicles for delivering drugs, genes, and cytokines in the treatment of muscular dystrophies including FSHD (Table 1). However, challenges such as poor serum stability, premature drug leakage, and clearance by the mononuclear phagocyte system (MPS) persist. Current strategies to overcome these include PEGylation, ligand conjugation and surface charge modulation.[51]

4 Nanotherapeutics in muscular dystrophy: lessons and prospects in FSHD

The application of nanocarriers in FSHD is barely explored. Hence, while considering nanotherapeutics for muscular dystrophies like FSHD, the development of a design to recognize regenerating muscle tissue markers or FSHD cues for improved precision and efficacy is important. In one study, when functionalized with muscle-homing ligands such as M12 peptides, A2G80, and ASSLNIA, NPs have demonstrated remarkable specificity in preclinical models, enabling the restoration of dystrophin or the targeted delivery of therapeutic cargo to diseased muscle tissues.[87,95,96] These smart delivery systems reduce the required therapeutic dose and limit off-target effects, an essential consideration for gene therapies, which are otherwise hindered by immunogenicity and inefficient systemic distribution when delivered *via* viral vectors like AAV. There is a prevalence of 70% anti-AAV in the population, and its immune recognition through the activation of capsid-specific CD4+ helper and CD8+ cells contributes to the immunotoxicity associated with AAV-based gene delivery.[97,98]

Besides the utmost importance of the muscle specificity, nanotherapeutic strategies for FSHD must also consider the disease-associated microenvironment, including changes in the ECM, oxidative stress, inflammation, immune cell infiltration, and impaired myogenesis and muscle regeneration. Nanoparticle functionalization strategies like ECM-degrading enzymes (*e.g.*, bromelain) and TGF-β pathway inhibition offer promising solutions to overcome stromal hindrances and improve nanoparticle penetration, retention, and therapeutic impact.[99,100]

To mitigate the effects of inflammation, macrophage polarization can be one such approach. Macrophages are present in abundance at the site of muscle regeneration. Whenever there is muscle injury, monocytes differentiate into pro-inflammatory Ly6C + M1 macrophages. Moreover, this attracts additional monocytes, enhances the removal of cellular debris, and promotes the transition of M1 macrophages into anti-inflammatory Ly6C − M2 macrophages, which promote the proliferation of muscle progenitor cells.[101] An imbalance in the switch from M1 to M2 macrophages can disrupt the muscle repair process. Studies show that AuNPs, because of their optimum shape, size, and surface modification, can modulate M2 macrophage polarization to promote tissue healing and regeneration through pathways like p38 MAPK.[102] Similarly, zinc-dihydromyricetin (Zn-DHM) nanozyme TiO_2 nanotubes and CeO_2 nanoparticles have been shown to support macrophage transition from the pro-inflammatory M1 state to the regenerative M2 state, promoting muscle regeneration and increasing anti-inflammatory markers.[103–107]

In muscular dystrophies like FSHD, excessive production of reactive oxygen species (ROS) can trigger inflammation and worsen the disease. Wang Zhicun *et al.* designed ROS-responsive polymer nanoparticles thioketal–dopamine loaded with the anti-inflammatory drug dexamethasone and combined them with a reversible ECM-like hydrogel.[108] This was tested in MI and stroke models. The results showed that the hydrogel effectively reduced ROS levels and inflammation *in vitro* and *in vivo*. It reduced inflammation, decreased cell death, and supported new blood vessel formation, demonstrating its therapeutic potential for FSHD.

Exploring cell membrane-biomimetic nanoparticles could open new possibilities for FSHD therapy. These NPs are derived from natural cell membranes and mimic the features and functions of native cells, which helps them to evade immune clearance. Overall, this composition improves target specificity and biocompatibility, reduces immunogenicity, and increases circulation time in the bloodstream. Common

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Table 1 Published studies (up to 2025) highlighting diverse nanocarriers, their therapeutic cargos, and preclinical outcomes, providing an overview of different nanoformulations and their translational potential in MDs

Nanoparticle type (year)	Cargo/therapeutic agent	Experimental model	Target/application	Reference
PEG-nano-liposomes (2019)	Methylprednisolone hemisuccinate (MPS)	MDX mice (DMD model)	Diaphragm muscle	60
Nanolipodendrosome (2013)	MyoD and myogenin	SW1 dystrophic mice, C2C12 myoblasts	Myogenic differentiation	86
Hybrid liposomes (DMPC + polyoxyethylene lauryl ether) (2011)	Gentamicin	mdx mice	Drug delivery	59
Guanidinium-rich lipopeptide NPs (2023)	CRISPR-Cas9/sgRNA RNP	Ai14 and mdx mice	Gene editing	82
Hydroxyl-PAMAM dendrimer (2017)	Angiotensin (1–7)	C57BL/10J male mice	Anti-fibrotic therapy	64
Nanobubbles (2021)	PMO AON (DUX4 suppression)	FSHD cell model	Antisense delivery	77
G5-PAMAM (2019)	Plasmid DNA + skeletal muscle-targeted peptide	C2C12 cells, BALB/c mice	Muscle-targeted gene delivery	87
Hyperbranched PEAs (2012)	Plasmid DNA	mdx mice, CHO, C2C12, HSK cells	Gene delivery	88
LNPs (2020)	CRISPR-Cas9 RNP	DMD and C57BL/6 mice	Gene editing	81
Nanocapsules (2019)	Cas9/sgRNA RNP	HEK293 cells, mouse retinal pigment epithelium, and skeletal muscle	Intracellular delivery/ gene editing	89
SMNP (2021)	Cas9 + dual sgRNAs	AC16, iPSCs, MSCs	Gene editing	58
Gold NPs (2017)	Cas9 RNP + donor DNA	mdx mice	IM gene editing	83
Biomineralized SpRY NPs (2022)	Cas9 (SpRY variant)	C2C12, HEK293, BALB/c mice	Gene editing	84
Mesoporous silica NPs (2023)	Near-infrared (NIR) fluorescent dyes/proteins	mdx mice	Biodistribution	70
PEGylated gold NPs (2021)	IL-4 or IL-10 conjugates	Primary T cells, mdx mice	Anti-inflammatory response	62
PLGA nanospheres (2009)	PEI–PEG–oligonucleotide complex	mdx mice	Sustained oligo release	75
Lipid NPs (2025)	miR-130a (PPARG regulator)	HFD C57BL/6J mice	Skeletal muscle metabolism	90
SORT LNPs (2025)	Cas9 cargo	LGMDR7 mouse model	Gene correction	91
LNPs (2025)	Emerin mRNA	H2K WT and EMD–/y myogenic progenitors	Protein replacement	92
PLGA NPs (2025)	AMPK activator (compound 991)	D2-mdx mice	Gastrocnemius and diaphragm	93
AUNPs (2025)	miRNA	D2-mdx mice	Muscle regeneration	76
LNPs (2025)	WNT7A mRNA	FAPs, C2C12, mouse injury model	Muscle hypertrophy	65
Arginine-modified hydroxyapatite NPs (2025)	Dystrophin gene	Myotubes and patient-derived cells	Gene delivery	69
DU01 conjugated lipid docosanoic acid (DCA) NPs (2025)	DUX4-targeting siRNA	FSHD patient-derived myotubes *ex vivo*	Gene silencing	94
Superparamagnetic iron oxide nanoparticles (SPIONs)(2025)	Deflazacort and ibuprofen	mdx and C57BL/10 mice	Systemic anti-inflammatory delivery	58

sources for such biomimetic membranes include erythrocytes, platelets, neutrophils, macrophages, mesenchymal stem cells, and cancer cells.[109] In a recent study, muscle-homing peptides were used to develop biomimetic curcumin nanoparticles (M12MNCs).[110] These nanoparticles showed the increased solubility and bioavailability of curcumin and additionally improved muscle dysfunction in aging mice by regulating the SphK1/Spns2/S1PR2 axis. This refers to a molecular signaling pathway that controls inflammation, cell survival, and other physiological processes in tissues like skeletal muscles. In another study, exosome-mimetic nanocarriers derived from synthetic vesicles for gene therapy in neurodegenerative diseases mimicked natural exosomes for delivering siRNA across the blood–brain barrier, emphasizing the therapeutic potential of such designs.[111]

Although biomimetic nanoparticles can evade immune recognition, they can still be recognized by the MPS. This occurs primarily due to the formation of a protein corona and the progressive loss of masking of functional membrane proteins. Therefore, repeated administration and long-term exposure assessments are still required.[112,113]

Besides these advances, artificial intelligence and machine learning are also being integrated into nanoparticle design pipelines, enabling predictive modeling of NP interactions with cells, organs, and immune components, thereby accelerating the development of personalized nanomedicines.

5 Conclusion

From the earlier discovery of the enhanced permeability and retention (EPR) effect of nanoparticles to the approval of nanomedicines such as Doxil® and Telisotuzumab vedotin (Teliso-V), nanoparticle-based therapies have revolutionized cancer treatment and are now being actively investigated for genetic muscular dystrophies.[114–116] Although nanoparticle platforms

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have advanced rapidly, their use in FSHD is still unexplored. Several critical shortcomings and priority areas for future work can be identified.

First, FSHD poses distinct hurdles, including the large, heterogeneously affected distribution of skeletal muscles, the lack of hyperpermeable vasculature, and the inherently poor biodistribution of systemically delivered nanocarriers to muscle tissue.[70] While mesoporous silica nanoparticles have shown preferential accumulation in regenerating fibers in DMD, comparable strategies have not been systematically evaluated in FSHD.[117] Consequently, there is a pressing need for muscle-directed targeting motifs such as those engaging ICAM-1 or markers of regeneration to improve precision and therapeutic yield.[118] Second, current nucleic acid delivery approaches for suppressing DUX4 remain suboptimal. Although antisense oligonucleotides, siRNAs, and CRISPR–Cas systems are under active investigation, recent work with perfluoropentane nanobubbles despite efficient loading of PMO-AONs did not achieve DUX4 knockdown. Next-generation carriers should therefore prioritize robust endosomal escape (*e.g.*, *via* pH-responsive polymers or proton sponge mechanisms), effective nuclear delivery (for example, through NLS-conjugated constructs), and muscle-tropic vectors that combine *in vivo* stability with stimuli-responsive release profiles.[119] Proof-of-concept studies in DMD such as gold nanoparticles delivering CRISPR components with low off-target activity highlight the feasibility of such designs.[83]Third, the formation of a protein corona in biological fluids can drastically remodel nanoparticle surfaces, reshaping biodistribution, clearance, immunogenicity, and target engagement, and introducing substantial unpredictability that impedes translation.[120] Rational surface engineering using PEGylation, since PEG chains create steric hindrance that reduces protein adsorption, and zwitterionic coatings such as poly(carboxybetaine), poly(sulfobetaine), and phosphorylcholine resist non-specific protein adsorption and biofouling due to their strong hydration *via* ionic solvation.[121] In contrast to chemical coating, biomimetic membranes integrated with proteins, such as CD47 and CD45, on the surface of NPs to signal a "self-marker" message to circulating monocytes, are being pursued to dampen corona effects and stabilize *in vivo* behavior.[122–124]

Other major concerns are immune activation, off-target accumulation, and crossing sensitive barriers such as the blood–brain barrier and the placenta, raising systemic safety questions.[125,126] For example, inorganic NPs, such as silver nanoparticles (AgNPs), showed significant ROS generation, DNA damage, and organ accumulation at concentrations ≥20 mg kg^{-1}.[127] Similarly, with AuNPs, ROS-mediated cytotoxicity was observed at higher concentrations with smaller sizes (~1 mM and 6.2–24.3 nm), while accumulation of larger particles (~40–60 nm) in the liver and spleen persisted for up to 90 days.[128] Hence, thorough *in vivo* assessment and whole-body imaging (PET, MRI) are essential to map distribution, residence, and clearance.[129,130] However, some of these green-synthesized NPs (AgNPs) have been found to be less toxic in environmental settings due to the formation of stable aggregates upon interaction with natural organic matter, which simultaneously reduces toxicity by releasing less ROS (Ag^{+}).[131] Whether this can be extrapolated to the behavior of such nanoparticles when used as therapeutics needs to be investigated.

Notably, long-term safety in the context of chronic muscle disease remains poorly defined. Scaling and clinical translation pose practical bottlenecks. Complex, multistep synthesis complicates good manufacturing practice, hinders scale-up and contributes to batch variability.[132] Although the regulators emphasize vigorous quality control, standards for reporting its physicochemical attributes and biological performance are still evolving in academic settings.[133] There is a lack of standardized evaluation criteria and guidelines for nanomedicines

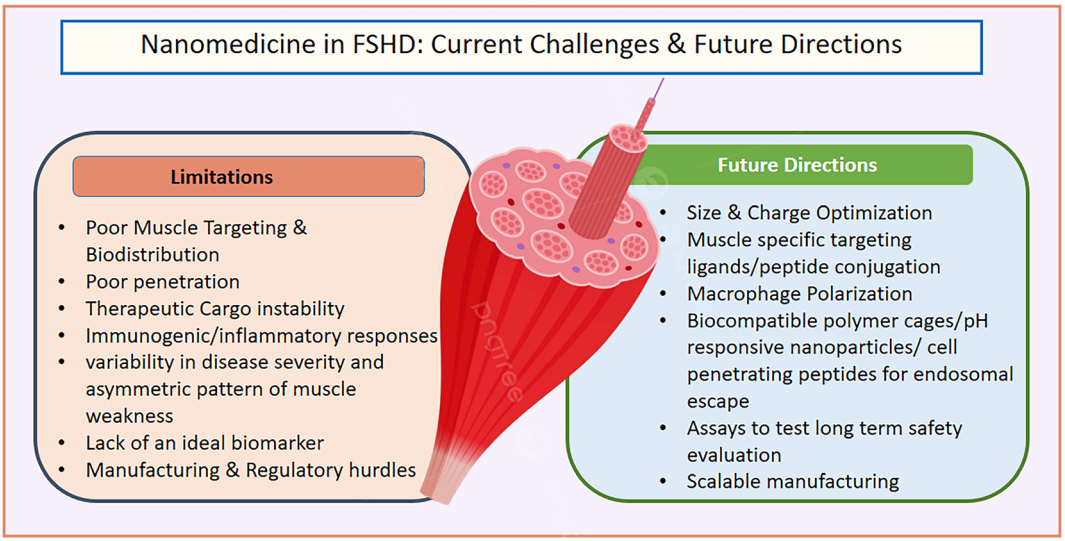

Fig. 4 Current challenges and translational limitations of nanoparticle-based therapeutics in FSHD.

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compared to those for small-molecule drugs, due to variability in size and surface properties. Additionally, these require studies on immunogenicity, biodistribution, and long-term accumulation. Thus, regulatory agencies like the FDA and EMA require the evaluation of each nanoparticle individually.[134,135] Initiatives like the European Nanomedicine Characterization Laboratory (EUNCL) offer comprehensive pipelines that consider physical, chemical, and biological testing, but broader implementation is needed to standardize the field.

In addition, there are still significant concerns about the handling of nanomaterials and the moral implications of gene-editing technologies such as CRISPR. Furthermore, policies differ across jurisdictions and lack full harmonization.[136] Thus, the development of ethical frameworks is essential as nanomedicine expands beyond oncology into hereditary diseases such as FSHD.

In conclusion, a multidisciplinary approach that integrates computational modeling, high-throughput screening, and multi-omics data to rationally design nanocarriers tailored to the FSHD muscle microenvironment can offer unprecedented solutions (Fig. 4). Nevertheless, there are certain drugs that have been approved recently in clinical trials – mRESVIA (mRNA-1345)(NCT07117487, NCT05127434), which shows a promising future of nanodrugs in the treatment of FSHD by providing new avenues for more efficient and targeted therapies. Moreover, to fully realize the potential, ensure biosafety, and achieve long-term biocompatibility, extensive research is necessary.

Author contributions

Deepali Shukla: conceptualization; investigation; and writing – review and editing. Meenal Kowshik: editing (equal) and reviewing the article. Indrani Talukdar: reviewing the article.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability

No new data were generated or analysed as part of this review. All information discussed in this article is derived from the previously published studies, which are cited throughout the article.

Acknowledgements

We thank the University Grants Commission–Council of Scientific and Industrial Research (CSIR), India, Grant No. 211610155029 and BFI/BITS Collaborative Grants BFI/24/114 for financial support and BITS-Pilani, K. K. Birla Goa Campus, India for the infrastructure. The authors express their thanks and gratitude to Prof. Bharat Bhushan (The Ohio State University, San Jose, CA) for his insights and to the 32nd FSHD IRC 2025 organizers for providing data on recent progress in FSHD therapeutics.

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