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Musculoskeletal ultrasound-guided cellular therapy: Current applications and future directions in skeletal muscle regeneration

Yu Chunfeng [1, 2, 3], Li Xueqin [1, 2, 3], Zhao Wen [1, 2, 3], Zhou Lingyan [1, 2, 3*]

[1] Department of Ultrasound Medicine, Minda Hospital of Hubei Minzu University, Enshi 445000, China
[2] Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases, Minda Hospital of Hubei Minzu University, Enshi 445000, Hubei, China
[3] Hubei Provincial Clinical Medical Research Center for Nephropathy, Minda Hospital of Hubei Minzu University, Enshi 445000, Hubei, China

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ABSTRACT

Skeletal muscle exhibits robust intrinsic regeneration after acute injury, yet severe, chronic, or age-related damage commonly culminates in fibrosis, fatty infiltration, and lasting functional impairment. This review integrates the cellular and molecular biology of successful healing spanning inflammation resolution, satellite-cell activation, fibro-adipogenic progenitor regulation, angiogenesis, and extracellular-matrix remodeling with the key failure modes that cellular therapies seek to overcome. It evaluates an expanding portfolio of platforms (mesenchymal stromal cells, satellite cells, iPSC-derived myogenic progenitors, exosomes, secretome, and biomaterial scaffolds) that act primarily through paracrine, immunomodulatory, and differentiative mechanisms, while acknowledging persistent translational barriers of low engraftment, immunogenicity, and scalability. Musculoskeletal ultrasound is positioned as a pivotal enabling technology: high-frequency B-mode, Doppler, elastography, and contrast-enhanced imaging provide real-time lesion mapping, vascular targeting, injectate tracking, and non-invasive longitudinal biomarkers of perfusion and stiffness. Sonoporation further enhances cell/exosome uptake and homing. Preclinical studies consistently show 2-5-fold gains in retention and functional recovery with ultrasound-guided versus blind delivery; clinical evidence, though still early-phase, reports acceptable safety and modest signals of benefit in sarcopenia, volumetric muscle loss, and Duchenne dystrophy. This review highlights how ultrasound-guided delivery, combined with optimized cellular platforms and biomaterials, addresses key translational barriers and proposes a realistic 2025-2030 roadmap centered on standardization, AI navigation, personalized hypoimmunogenic products, synergistic combinations (cells+biomaterials+sonoporation), and adequately powered RCTs with quantitative imaging endpoints. When these milestones are achieved, ultrasound-guided cellular therapies will offer a transformative solution for conditions in which endogenous repair fails.

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Introduction

Skeletal muscle injuries and degenerative conditions represent a significant global health challenge, affecting millions worldwide and imposing substantial economic and functional burdens. Acute traumas, such as strains, lacerations, and volumetric muscle loss (VML), often result from sports, accidents, or military injuries, leading to immediate functional impairment and prolonged recovery periods. Chronic and age-related degeneration, including sarcopenia and muscular dystrophies like Duchenne muscular dystrophy (DMD), exacerbate this issue by causing progressive muscle wasting, fibrosis, and fatty infiltration. For instance, sarcopenia affects up to 50% of individuals over age of 80, contributing to frailty, falls, and reduced quality of life (1-3). Muscular dystrophies, such as DMD, lead to early mobility loss and life expectancy reduction, with current treatments like corticosteroids providing only symptomatic relief without addressing underlying regeneration failures (4, 5). Limitations of existing therapies, physical rehabilitation, or surgical reconstruction—include incomplete functional restoration, high re-injury rates (up to 30% in athletes), and persistent deficits in strength and range of motion (6-8). Economically, these conditions strain healthcare systems; VML alone incurs billions in annual costs due to prolonged disability and rehabilitation, while sarcopenia contributes to over $40 billion in U.S. healthcare expenditures through associated comorbidities like osteoporosis and cardiovascular disease. Functionally, patients face lasting impairments, with studies showing only 37% strength recovery in human VML cohorts treated with acellular scaffolds and therapy (8). These burdens underscore the urgent need for interventions that enhance endogenous repair mechanisms, particularly in contexts where native regeneration fails due to excessive inflammation, vascular insufficiency, or stem cell decline [2.2 Why Regeneration Fails].

***Corresponding author:** Zhou Lingyan. Department of Ultrasound Medicine, Minda Hospital of Hubei Minzu University, Enshi 445000, China, Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases, Minda Hospital of Hubei Minzu University, Enshi 445000, Hubei, China, Hubei Provincial Clinical Medical Research Center for Nephropathy, Minda Hospital of Hubei Minzu University, Enshi 445000, Hubei, China. Email: Lingyan.zhou100@gmail.com

The emergence of regenerative cellular therapies offers a paradigm shift by targeting these failure modes through paracrine signaling, immunomodulation, and direct tissue integration. Preclinical promise is evident: MSCs restore up to ~110% of contralateral force in analogous preclinical laceration models, while exosomes accelerate regeneration by 26% in meta-analyses (9, 10). However, inconsistent clinical outcomes highlight limitations. These discrepancies arise from poor scalability, immunogenicity, and heterogeneous protocols, with effect sizes rarely exceeding 0.5 and placebo responses confounding results (7.4 Critical Appraisal of Clinical Evidence). Critically, while paracrine effects dominate (e.g., IGF-1-driven M2 transition and VEGF-mediated perfusion), long-term integration remains elusive, emphasizing the need for optimized delivery to maximize therapeutic impact [6.3 Mechanisms of Regeneration].

Delivery method critically determines therapeutic success, as low cell survival (<5% without support), imprecise targeting, and inadequate retention/engraftment undermine efficacy. Intramuscular or systemic administration often results in backflow, rapid clearance, and off-target deposition, with <1-5% long-term survival in non-guided models due to hypoxia, inflammation, and mechanical shear (11, 12). Precision is essential for homing to regenerative niches—e.g., perivascular zones for angiogenesis or intralesional sites for paracrine activation of resident MuSCs and macrophages (13, 14). Enhanced retention (2-5-fold) through biomaterials like hydrogels or fractionated injection correlates directly with superior histological outcomes, such as increased centrally nucleated fibers and reduced fibrosis (15-18). Engraftment barriers, including anoikis and immune rejection, are mitigated by prosurvival factors or preconditioning, yielding >45% more donor-derived fibers (19, 20). Yet, blind injections achieve only 11-80% accuracy, increasing risks of neurovascular injury and suboptimal distribution (21, 22). This underscores that without targeted delivery, even potent cellular platforms fail to bridge biological gaps like persistent TGF-β signaling or vascular insufficiency [2.3 Rationale for Cellular Therapy].

Musculoskeletal ultrasound (US) emerges as a pivotal tool to address these challenges, providing real-time, radiation-free guidance that enhances precision and monitors outcomes. High-frequency B-mode (6-70 MHz) visualizes fascicular disruptions and lesions with 30 µm resolution, while Doppler and contrast-enhanced ultrasound (CEUS) map perfusion for vascular-targeted delivery (23-27). Elastography quantifies stiffness as a fibrosis surrogate, correlating strongly with histology (r=0.68-0.87)(28, 29). Compared to MRI or CT, ultrasound offers superior accessibility (25% cost of MRI), shorter procedures, and equivalent accuracy (95-100% vs 72-82% blind) without contraindications or radiation (30-37). Increasing adoption in regenerative interventions is evidenced by 2-5-fold retention gains in preclinical guided vs non-guided studies, with clinical series reporting faster athlete recovery and <0.5% complications (21, 22, 38-40). Advanced features like 3D volumetrics (ICC>0.97) and AI-assisted navigation (88.8% structure identification) further augment operator consistency (41-46). No comprehensive review, to our knowledge, has yet integrated musculoskeletal US technology with cellular therapy delivery strategies, biological mechanisms, and clinical translation in skeletal muscle regeneration.

This review aims to provide a critical synthesis of these elements, evaluating how ultrasound-guided approaches overcome biological bottlenecks (e.g., inflammation resolution and MuSC activation) through mechanistic insights from preclinical models, while identifying translational barriers like protocol heterogeneity and manufacturing costs. By contrasting guided vs non-guided outcomes and proposing a 2025-2040 roadmap, it highlights opportunities for synergistic integration (cells+biomaterials+sonoporation) to achieve transformative clinical impact [9.5 Roadmap to Clinical Adoption].

Biology of skeletal muscle regeneration

Skeletal muscle exhibits a robust intrinsic regenerative capacity that restores structure and function after injury in most acute scenarios. This process depends on a highly orchestrated interplay among immune cells, muscle stem cells (satellite cells, MuSCs), fibro-adipogenic progenitors (FAPs), vascular cells, and the extracellular matrix (ECM). However, regeneration frequently fails in severe, chronic, or aged contexts, resulting in fibrosis, fatty infiltration, and persistent functional deficits. Understanding the native healing cascade, its failure modes, and the mechanistic gaps that cellular therapies aim to bridge is essential for developing targeted interventions.

Native muscle healing cascade

The regenerative process unfolds in overlapping phases—inflammation, satellite cell activation, and remodeling—typically completing within 3-4 weeks in young rodents and humans after mild injury (see Figure 1 for a schematic overview of these phases and their timelines). Failure of these coordinated processes in severe, chronic, or aged muscle frequently leads to persistent inflammation, impaired MuSC function, excessive fibrosis, and incomplete vascularization (as highlighted in Figure 1).

Why regeneration fails

Fibrosis Severe or chronic injury leads to excessive ECM accumulation that replaces functional muscle (refer to Figure 2 for visualized failure points, such as persistent TGF-β signaling leading to fibrosis). Persistent TGF-β signaling is central: it inhibits MuSC activation/differentiation while driving FAPs toward myofibroblasts via SMAD3 and YAP/TAZ (47-50). Myeloid-derived TGF-β1, PDGFRα-mediated myofibroblast survival, and periostin-enhanced fibroblast migration amplify fibrosis and antagonize satellite cell proliferation (51-54). Fibroblast-MuSC interactions are phenotype-dependent; pro-fibrotic fibroblasts exacerbate inflammation and scarring, while regenerative phenotypes initially support repair (55, 56).

Vascular insufficiency: Aging markedly reduces VEGFA levels, limiting myofiber CSA despite preserved capillary density (57,58). Endothelial Notch ligand (Dll4) signaling maintains MuSC quiescence, but chronic vascular niche dysfunction impairs activation and capillarization (59-61) Ischemia creates a hostile microenvironment, causing MuSC death, premature differentiation, and incomplete regeneration (62).

Aging stem cell decline: Aging reduces MuSC numbers and function through cell-intrinsic senescence (p53/p21, p16INK4a/pRB; autophagy failure; AMPK/p27Kip1 impairment) and extrinsic niche changes (ECM stiffening, chronic inflammation, elevated PAI-1, and senescent

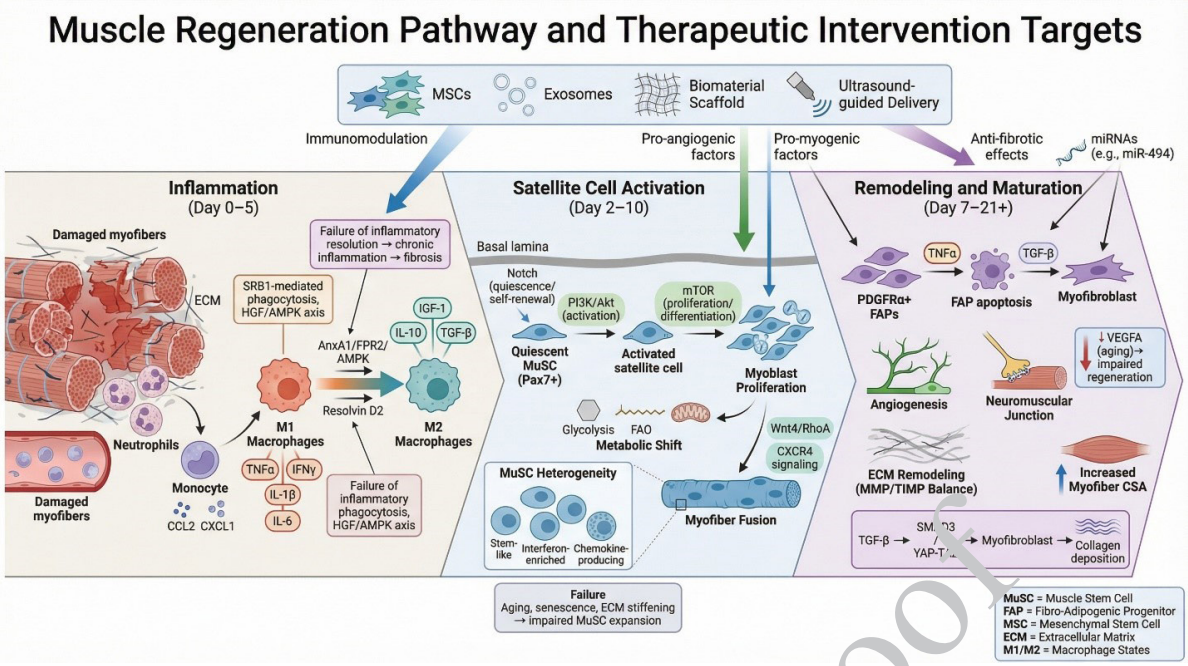

Figure 1. Schematic representation of skeletal muscle regeneration post-injury, showing phases: inflammation (Days 0-5) with immune recruitment/resolution; satellite cell activation (Days 2-10) via Notch, PI3K/Akt, mTOR; remodeling/maturation (Days 7-21+) with ECM turnover, angiogenesis, myfiber fusion, and neuromuscular junctions. Highlights failure modes (e.g., fibrosis, aging, and vascular issues) and therapeutic targets (MSCs, exosomes, scaffolds, ultrasound delivery, pro-angiogenic/myogenic factors, and anti-fibrotic miRNAs)

cell accumulation)(63-71). These alterations promote fibrogenic conversion of MuSCs and a pro-inflammatory microenvironment that arrests proliferation(72-74).

Rationale for cellular therapy

Cellular therapies target these bottlenecks through paracrine signaling, myogenic differentiation, and immunomodulation (as illustrated in Figure 1, with intervention points like MSCs, exosomes, and anti-fibrotic effects). These mechanisms are detailed in Section 3.

Collectively, these biological insights reveal that successful regeneration requires precise temporal control of inflammation, MuSC expansion/differentiation, vascular support, and ECM remodeling. Cellular therapies are rationally positioned to restore these processes where endogenous mechanisms fail, providing the foundation for ultrasound-guided delivery strategies discussed in subsequent sections.

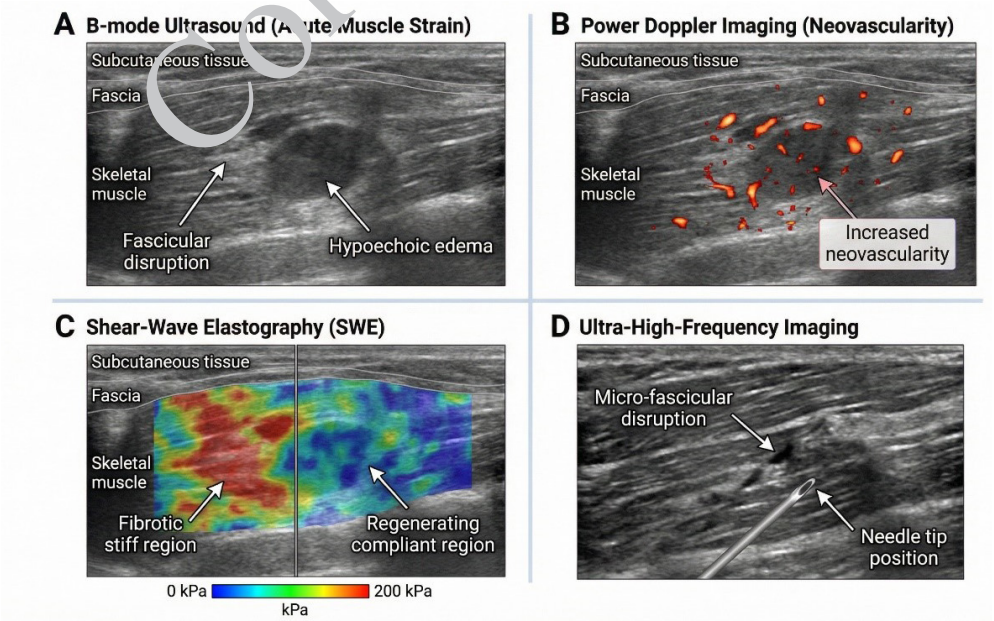

Figure 2. Ultrasound imaging features of injured skeletal muscle in regenerative medicine
(A) B-mode ultrasound showing acute muscle strain with fascicular disruption and hypoechoic edema. (B) Power Doppler imaging demonstrating increased neovascularity. (C) Shear-wave elastography (SWE) map highlighting fibrotic stiff regions (red) versus regenerating compliant regions (blue/green), with stiffness scale in kPa. (D) Ultra-high-frequency imaging revealing micro-fascicular disruption and needle tip position for precise delivery

Cellular therapy platforms for muscle regeneration

Building on the biological mechanisms of muscle regeneration and its failure modes outlined previously, a wide array of cellular, cell-free, and biomaterial-based platforms have been developed to restore function in acute injury, VML, sarcopenia, and dystrophies. These strategies address key bottlenecks—poor endogenous stem cell function, excessive fibrosis, vascular insufficiency, and hostile microenvironments—by supplying reparative cells, paracrine signals, or structural support. While preclinical data are promising, translational hurdles including cell survival, immunogenicity, scalability, and regulatory compliance persist. This section critically examines stem/progenitor cell types, cell-free approaches, delivery vehicles, and their comparative performance (summarized in Table 1), with particular emphasis on how these platforms can be optimized for ultrasound-guided delivery.

Stem and progenitor cell types

Mesenchymal Stem Cells (MSCs): MSCs (bone marrow-, adipose-, umbilical cord-, or perinatal-derived) primarily exert therapeutic effects through paracrine and immunomodulatory actions rather than direct myogenic differentiation. In rodent laceration, crush, and VML models, bone marrow MSCs reduce fibrosis while increasing regenerating myofibers and contractile force recovery (75, 76). Allogeneic BM-MSCs accelerate force restoration (day 14: 110% vs ~80-88% in controls) and lower fibrosis indices in acute laceration (9).

Survival and retention remain major limitations (<5% long-term engraftment in most intramuscular studies), exacerbated by inflammatory/hypoxic microenvironments that impair viability, nitric oxide production, M1→M2 transition, and angiogenic capacity (11). Hypoxic preconditioning or prosurvival factors can prolong retention (77).

Source-specific differences are modest: bone marrow, adipose, and umbilical cord MSCs yield comparable muscle mass and cross-sectional area (CSA) gains in atrophy models, though adipose-derived MSCs may reduce collagen deposition more effectively in acute laceration (78). Preclinical efficacy is documented in sarcopenia (restored strength via satellite cell activation and autophagy) and VML (improved mass, MyoD/myogenin expression, centrally nucleated fibers when delivered in spheroids or constructs)(79, 80). Clinical evidence for skeletal muscle remains sparse; only acellular scaffolds plus physical therapy have reached human VML trials with modest gains (81).

Satellite Cells (MuSCs): Autologous or expanded MuSCs offer direct myogenic potential but face practical constraints. Synthetic hydrogels with optimized stiffness (matching native muscle), RGD ligands, and degradability markedly improve MuSC survival, proliferation, colony size, and engraftment in aged/dystrophic muscle compared with collagen or cell-only controls (82). MuSCs reciprocally signal with vasculature (VEGFA recruits endothelium; Dll4/Notch maintains quiescence) and immune cells (neutrophils/macrophages supply cytokines for activation; Tregs modulate phenotype)(59, 83).

Induced Pluripotent Stem Cells (iPSCs) and Pericytes:

Table 1. Comparative overview of cellular, cell-free, and biomaterial platforms for skeletal muscle regeneration

Cell Type/Platform	Mechanisms	Advantages	Limitations	References
MSCs (BM, adipose, UC)	Paracrine: IGF-1, VEGF, cytokines; immunomod: ↓ IL-1β, IL-6/TNF-α/TGF-β, M1→M2; minor differentiation	Versatile; ↓ fibrosis, ↑ myogenesis/angiogenesis; force recovery ~110%; effective in sarcopenia/VML	Engraftment <5%; hypoxia/inflammation impair survival; modest gains (2-7%); high costs	(9, 11, 75-81, 94)
Satellite Cells (MuSCs)	Direct myogenic diff.; quiescence exit via Wnt/Notch/cues; signal with vasc./immune	Autologous; aging reversible; hydrogels ↑ engraftment/survival/prolif	Expansion constraints; aging defects; limited clinical data	(59, 82, 83, 95, 96)
iPSCs-derived iMPs	Direct diff./maturation *in vivo*; repopulate niche; engineered for migration/payloads	High diff. potential; VML recovery with scaffolds; multitasking	Preclinical; tumorigenicity; immunogenicity; incomplete diff.; no trials	(84, 85)
Pericytes	IGF-1/ANGPT1 myogenesis/myotube/vasc. stab.; ↑ angio vs MSCs	Hypoxia robust; restore CSA/capillaries; synergistic	No clinical data; ablation → hypotrophy	(86, 87)
Exosomes/Secretome	miRNAs (miR-494/126-3p/myomiRs), factors (IGF-1/HGF/FGF-2); Wnt/PI3K/Akt/MAPK; M2 polar.; ↓ inflam./fibrosis	Efficacy ~cells (26%); safer (no tumor/immuno); easy scale/storage; IM retention better	No long-term engraft.; standardization needed; IV poor tropism	(10, 88, 89, 97-101)
Biomaterials (Hydrogels/Scaffolds/Nanocarriers)	ECM mimic, release control, mech. match; ↑ retention/survival; promote prolif./diff	↑ donor fibers 3-45%; +65% myofiber, +80% force; 3-10x factor bioavail.; anisotropic support	Efficacy/safety/practical trade-offs; toxicity varies; dynamic integration complex	(15, 16, 90-93, 102)

Human iPSC-derived myogenic progenitors (iMPs) remain preclinical but show rapid progress in purification, maturation, and engineering. Transplanted iMPs mature *in vivo*, repopulate the satellite niche, and support secondary regeneration; hybrid scaffold constructs achieve functional recovery in VML (84). Engineering strategies now confer enhanced migration (DLL4/PDGF-BB), therapeutic payloads, or multitasking functions beyond native capacity (85). Major barriers include tumorigenicity from residual pluripotent cells or acquired genetic/epigenetic aberrations, immunogenicity, and incomplete *in vivo* differentiation (84). No clinical muscle trials have succeeded to date.

Pericytes (type-1/2 subpopulations) contribute to repair via IGF-1/ANGPT1-mediated myogenesis, myotube formation, and vascular stabilization. Pericyte ablation causes hypotrophy; transplantation restores CSA and capillary-to-fiber ratios post-atrophy (86). In ischemic models they outperform MSCs in angiogenesis while synergizing with other progenitors (87). Their robustness in hypoxia makes them attractive, though clinical data are absent. Comparative advantages and limitations of these cell types relative to others are summarized in Table 1.

Cell-free approaches

Exosomes and secretome therapy: MSC-derived exosomes and secretome recapitulate many whole-cell benefits without engraftment risks. Intramuscular injection provides superior local retention versus intravenous (poor muscle tropism; liver/spleen/lung dominance)(88).

Secretome shows comparable efficacy to MSCs in meta-analyses of animal models (~26% improvement in tissue outcomes) with advantages in safety (no tumorigenicity, minimal immunogenicity, and easier storage/scale-up)(10). Manufacturing standardization, GMP purification, and heterogeneity remain major barriers to reproducibility and regulatory approval (89).

Biomaterials and delivery vehicles

Hydrogels: Injectable and composite hydrogels provide ECM-mimetic cues, controlled release, and mechanical matching (stiffness ~10-40 kPa optimal for myogenesis). They significantly enhance cell retention/survival versus bolus injection, promote myofiber density (+65%), contractile force restoration (up to +80%), and vascularized regeneration in VML (15, 16, 90). "Smart" self-healing or aligned variants further improve integration in mechanically demanding muscle (82).

Scaffolds and nanocarriers: Decellularized muscle matrices and 3D bioprinted/electrospun scaffolds better recapitulate muscle architecture and support maturation and vascularization (91, 92). Nanocarriers improve growth-factor bioavailability (3-10-fold) versus free injection (93). Integration of biomaterials with cellular or cell-free platforms addresses many limitations, and is particularly promising when combined with ultrasound-guided delivery, as outlined in the comparative overview in Table 1.

Comparative effectiveness and limitations

Direct head-to-head comparisons remain scarce, but patterns emerge. Cellular therapies (MSCs, MuSCs, and iMPs) provide both paracrine and differentiative contributions yet suffer low survival/engraftment (typically 1-2% without biomaterial support), operator variability, and high manufacturing cost/GMP demands. Cell-free exosomes/secretome offer similar functional gains with superior safety, stability, and scalability but lack long-term engraftment and require standardization. Pluripotent-derived cells carry higher tumorigenicity and epigenetic risks than adult MSCs or pericytes; allogeneic approaches raise immunogenicity concerns. Ethical issues center on iPSC reprogramming safety and source tissue acquisition. Cost and reimbursement barriers arise from complex *ex vivo* expansion, cryopreservation, and regulatory (ATMP) pathways. As summarized in Table 1, each platform trades off efficacy, safety, and practicality. These trade-offs underscore the value of hybrid approaches (cell + biomaterial) combined with ultrasound-guided delivery strategies explored in later sections. which can improve retention, reduce operator variability, and enhance clinical translatability.

Musculoskeletal ultrasound in regenerative medicine

Musculoskeletal US has become an indispensable tool in regenerative interventions for skeletal muscle, offering real-time, portable, radiation-free visualization that directly addresses the precision requirements of cellular and cell-free therapies. Its integration with regenerative platforms enables targeted delivery, immediate assessment of distribution, and longitudinal monitoring of healing, overcoming many limitations of alternative modalities.

Ultrasound physics relevant to regenerative delivery

High-frequency imaging provides the spatial resolution essential for visualizing injured muscle microstructure and guiding precise injections. Linear probes operating at 6-15 MHz are standard for superficial muscles, balancing penetration and detail, while ultra-high-frequency transducers (22-70 MHz) achieve resolutions down to 30 µm, enabling visualization of individual fascicles, micro-tears, and early fibrotic changes (23-25). Recent advances in transducers exceeding 20 MHz further enhance depiction of superficial muscle architecture and needle tip position during intralesional or perivascular delivery (103). Although standard 2-10 MHz arrays reliably detect acute strain-induced disorganization, higher frequencies improve pre-injection lesion mapping and post-delivery assessment of injectate dispersion (104)(Figures 2A and 2D).

Doppler vascular mapping identifies perfused regions and neovessels critical for cell survival and engraftment. Power Doppler reliably tracks spatiotemporal perfusion changes and shows excellent inter- and intra-rater reliability (ICC 0.86-0.95) for neovascularity (26, 105). However, CEUS often outperforms conventional Doppler in detecting incomplete microvascular recovery after injury, revealing persistent deficits when Doppler indicates normalization (27). Targeted Doppler-guided delivery remains underexplored in regenerative muscle contexts, yet vascular mapping intuitively improves homing of cells or exosomes to hypoxic or angiogenic niches (Figure 2B).

Elastography (strain and shear-wave) quantifies tissue stiffness, providing a surrogate for fibrosis versus regeneration. Shear-wave elastography (SWE) correlates strongly with histological collagen content (r=0.68-0.87) and MRI parameters (ρ≈0.82) in fibrotic, atrophic, and regenerating muscle (28, 29, 106). SWE stiffness reductions track functional gains in non-regenerative therapies (e.g.,

botulinum toxin and physical therapy), but its predictive value for stem-cell or exosome response is currently limited, with elastographic parameters showing only moderate correlations ($R^2 \leq 0.50$) to biochemical healing markers (107). Nonetheless, SWE offers non-invasive, repeatable monitoring of matrix remodeling after ultrasound-guided interventions (Figure 2C).

Advantages over other imaging modalities

Compared with MRI-guided injections, musculoskeletal US provides equivalent or superior procedural accuracy for most superficial targets while eliminating claustrophobia, contraindications, high cost, and long acquisition times (30, 76). Ultrasound is markedly more cost-effective (e.g., shoulder imaging ~25% the cost of MRI) and enables dynamic, real-time needle guidance without radiation (31). Direct head-to-head trials for regenerative muscle injections are scarce, but ultrasound consistently outperforms landmark-guided ("blind") techniques in accuracy (e.g., knee: 95.4% vs 82%; hip: 100% vs 72%) and yields superior short-term pain relief (32, 33, 108).

Versus CT-guided delivery, ultrasound achieves comparable precision for musculoskeletal injections while abolishing ionizing radiation exposure and reducing procedure time and needle passes (34-36). Meta-analyses of spinal and perineural injections show ultrasound lowers major adverse events (0.7% vs 6.5%) with equivalent therapeutic outcomes (37). No anatomical region has been identified where CT demonstrates superior accuracy, safety, or outcomes for soft-tissue regenerative delivery; ultrasound is therefore preferred when feasible.

Blind injections remain common but are markedly inferior in accuracy (often <70% for deep or complex targets) and carry higher risks of neurovascular injury or off-target deposition (21). Although accuracy gains with ultrasound do not always translate to superior long-term clinical outcomes in corticosteroid trials, the safety and precision advantages are especially relevant for cellular therapies, where even modest improvements in retention can substantially affect engraftment and paracrine efficacy (38).

Advanced ultrasound technologies

CEUS enables quantitative perfusion imaging and early detection of neovascularization after regenerative interventions. CEUS has tracked progressive vascularity increases after MSC/PRP treatment in bone nonunion ($P<0.001$) and shows comparable or superior sensitivity to MRI for perfusion deficits in muscle ischemia models (109, 110). Its ability to quantify microvascular blood volume and flow positions CEUS as a promising biomarker for engraftment success and functional recovery in future muscle regeneration trials.

3D ultrasound overcomes the slice-thickness limitations of 2D imaging, permitting accurate volumetric quantification of muscle mass, fascicle length, pennation angle, and regional strain (41-43). Freehand 3D acquisition with probe tracking achieves excellent reliability (ICC >0.97) and minimal detectable change (<5 ml), facilitating longitudinal assessment of regenerative outcomes without ionizing radiation (44).

AI-assisted targeting augments operator performance by automating anatomical landmark detection, optimizing probe positioning, and providing real-time needle trajectory guidance. In a prospective trial of rotator-cuff-interval injections, AI guidance reduced needle insertions, shortened procedure time, and improved pain relief and range-of-motion outcomes compared with manual ultrasound (SPADI 32.8 vs 44.5, $P=0.025$)(45). Broader musculoskeletal applications show AI increases correct structure identification (88.8% vs 77.4%) and overall procedural efficiency (46).

Collectively, these ultrasound technologies and their advanced variants establish a versatile, bedside platform that not only guides cellular delivery with unprecedented precision but also supplies immediate and longitudinal biomarkers of tissue response. This foundation directly enables the ultrasound-guided techniques examined in the following section.

Ultrasound-guided cellular delivery techniques

The success of cellular and cell-free therapies in skeletal muscle critically depends on accurate, reproducible delivery to the target microenvironment. Musculoskeletal US provides the spatial and temporal resolution required for real-time guidance, enabling strategies that maximize cell retention, minimize off-target deposition, and facilitate immediate assessment of injectate distribution. This section details precision injection approaches, technical optimizations, ultrasound-enhanced delivery methods, and the human factors that influence procedural consistency and outcomes.

Precision injection strategies

Intramuscular targeting remains the most common route for delivering MSCs, satellite cells, or exosomes into healthy or diffusely injured muscle. Under real-time ultrasound, cells are deposited into the muscle belly or fascial planes, often using a free-hand in-plane or out-of-plane technique with a 22-25 G echogenic needle. This approach achieves high local retention in rodent models (up to 20-30% at 24 hr when combined with biomaterials) and has been used successfully in early human trials for sarcopenia and post-traumatic atrophy (76, 79). Hydrodissection with small-volume saline or hyaluronate creates a temporary cleavage plane that improves cell spread while reducing pressure-related damage.

Intralesional injection targets the core of acute tears, contusions, or VML defects. High-frequency linear transducers (12-18 MHz) delineate the hypoechoic lesion, hematoma, or fibrotic scar, allowing precise deposition directly into the zone of injury. Intralesional delivery maximizes paracrine signaling to resident satellite cells and macrophages but risks uneven distribution if the lesion is heterogeneous; slow injection (0.1-0.2 ml/s) with real-time monitoring of hyperechoic injectate spread mitigates this (22, 111). Clinical series in athletes with grade II–III strains report faster return-to-play when PRP or MSCs are delivered intralesionally under ultrasound compared with perilesional or blind injection.

Perivascular delivery exploits the vascular niche that supports MuSC quiescence and homing. Color or power Doppler identifies intramuscular arterioles or the perimysial vascular network; cells or exosomes are injected adjacent to these vessels to promote rapid extravasation and engraftment. Preclinical data demonstrate that perivascular

MSC placement yields 2-3-fold higher retention and enhanced angiogenesis versus intramuscular bolus injection (13, 14). In large-animal ischemia-reperfusion models, perivascular delivery combined with focused ultrasound further increases cell homing via mechanotransductive signaling (112). This strategy is particularly promising for chronic conditions (sarcopenia and dystrophy) where vascular insufficiency limits endogenous repair.

However, when delivering cells suspended in viscous biomaterials such as hydrogels, the mechanical shear stress generated during needle passage must be carefully considered. High-viscosity vehicles can subject fragile progenitor cells to lethal hydrodynamic forces, potentially reducing post-injection viability even under precise ultrasound guidance. Optimized injection rates, larger-bore needles where feasible, and shear-protective formulations are therefore essential to preserve cell integrity in these hybrid approaches.

Technical optimization

Needle visualization is fundamental to safety and accuracy. Echogenic needles with laser-etched or polymer-coated tips produce bright specular echoes at angles up to 60° from perpendicular, enabling confident tip localization even in deep or anisotropic muscle (113). Compound imaging and needle-steering software further reduce tip obscuration. In-plane needle advancement parallel to the transducer footprint is preferred for long-axis visualization of the entire shaft and tip; out-of-plane techniques require "walk-down" confirmation or tip-tracking overlays.

Volume distribution critically influences therapeutic efficacy. Volumes >2-3 ml in a single intramuscular bolus frequently cause compartmental pressure spikes and backflow along the needle track. Fractionated injection (0.2-0.5 ml aliquots with needle repositioning) combined with hydrodissection produces more homogeneous spread, as confirmed by post-injection ultrasound or MRI (17). SWE can be used intra-procedurally to confirm that injectate has not created focal stiffness increases indicative of pressure necrosis.

Real-time monitoring allows immediate adjustment. Hyperechoic injectate (cells suspended in saline or contrast) is tracked as it disperses; persistent pooling prompts needle repositioning. Color Doppler confirms absence of intravascular injection, while CEUS or microbubble contrast can verify perfusion around the deposit site. These techniques have reduced inadvertent neurovascular injury to <0.5% in large registries of ultrasound-guided MSK procedures (Table 2)(39). The overall workflow for ultrasound-guided delivery, including patient positioning, probe selection, Doppler mapping, needle insertion, injectate spread monitoring, and post-procedure elastographic assessment, is illustrated in Figure 3.

Sonoporation and ultrasound-enhanced delivery

Sonoporation exploits acoustic cavitation to transiently permeabilize cell membranes and the vascular endothelium, dramatically increasing uptake of genes, exosomes, or nanoparticles without viral vectors. Low-intensity pulsed ultrasound (1 MHz, 0.5-2 W/cm², 20-50% duty cycle) combined with microbubbles (MBs) generates shear stress, micropores, and reactive oxygen species that create pores (50-500 nm) lasting seconds to minutes (115, 116).

In skeletal muscle, MB-assisted sonoporation has achieved 5–20-fold higher plasmid DNA transfection and exosome uptake compared with naked injection (116, 117). Targeted MBs conjugated with VEGF or SDF-1 further enhance site-specific homing of systemically administered MSCs (118). Preclinical rodent and porcine studies demonstrate accelerated force recovery, reduced

Table 2. Technical parameters in ultrasound-guided cellular delivery, including impacts on retention/engraftment, adverse events, and references

Parameter	Values/Recommendations	Impact on retention/engraftment	Impact on adverse events	References
Needle gauge	22–25 G echogenic (laser-etched or polymer-coated tips)	Improves needle visualization and tip localization; supports precise deposition for better cell spread and homing (2-3-fold higher retention in perivascular delivery)	Reduces neurovascular injury (<0.5% in registries); fewer needle passes	(39, 113)
Ultrasound frequency	High-frequency linear transducers: 12-18 MHz for intralesional; 6-15 MHz standard	Enhances lesion delineation and injectate monitoring; improves accuracy (74-96% vs 11-72% blind), leading to 2-5-fold retention gains	Minimizes off-target deposition and pressure necrosis; excellent safety profile	(21, 22)
Injection volume/rate	Volume: Fractionated 0.2–0.5 ml aliquots; avoid >2–3 ml bolus. Rate: 0.1-0.2 ml/s	Homogeneous spread; reduces backflow and clearance; up to 20-30% retention at 24 hr with biomaterials; correlates with superior histological outcomes	Prevents compartmental pressure spikes and necrosis; <0.5% complications with monitoring	(17, 39, 76, 79)
Hydrodissection use	Small-volume saline or hyaluronate to create cleavage plane	Improves injectate dispersion and reduces pressure damage; enhances local retention (2-5-fold vs bolus); supports paracrine signaling	Lowers risks of uneven distribution and shear injury; safer for heterogeneous lesions	(17, 111, 114)
Sonoporation parameters	1 MHz, 0.5-2 W/cm², 20-50% duty cycle; with microbubbles; MI < 0.5	5–20-fold higher exosome/plasmid uptake; increases homing and angiogenesis; accelerates force recovery and reduces fibrosis	Favorable at MI <0.5 (transient pores, minimal inflammation); acceptable in phase I trials	(115-121)

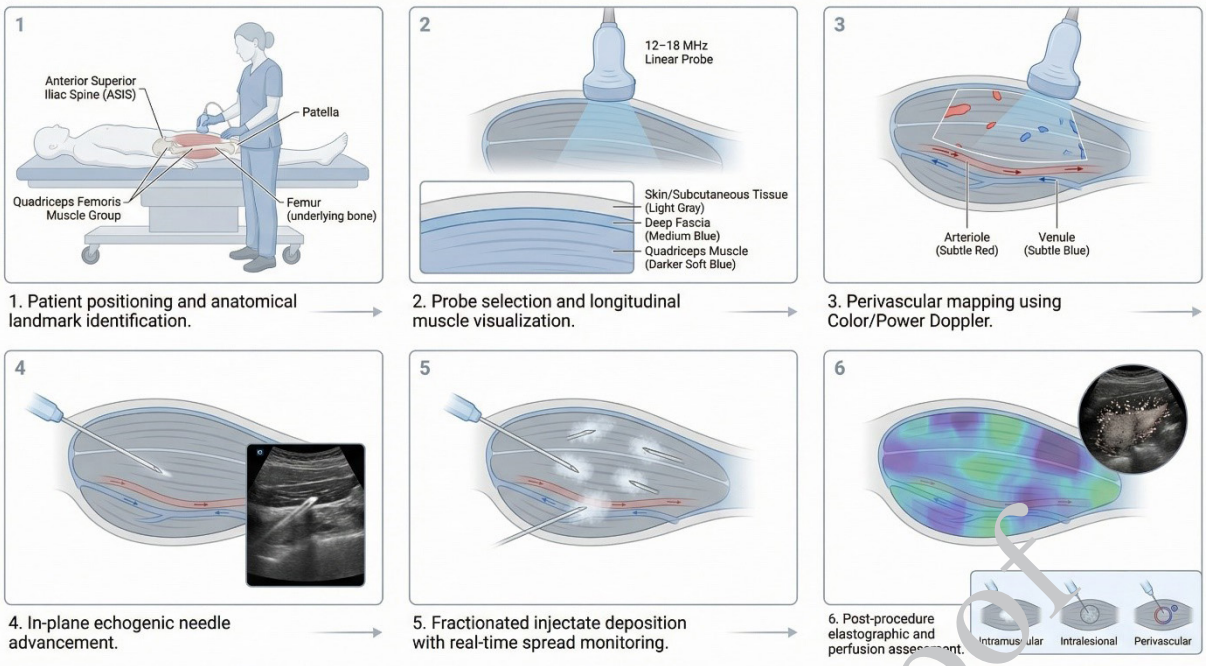

Figure 3. Step-by-step workflow for ultrasound-guided cellular delivery in skeletal muscle, showing patient positioning, probe selection, perivascular mapping, needle advancement, injectate deposition, and post-procedure assessment

fibrosis, and increased capillary density when sonoporation is applied immediately after intramuscular cell injection (119, 120). Safety profiles are generally favorable at clinically relevant pressures (<0.5 MPa), with transient membrane resealing and minimal long-term inflammation. Nevertheless, acoustic cavitation inherent to sonoporation carries a risk of cell membrane damage or cytotoxicity in progenitor cells if acoustic parameters (intensity, duty cycle, or microbubble concentration) are not precisely calibrated to the specific cell type and tissue context.

The technique is particularly synergistic with perivascular or intralesional delivery: focused ultrasound (FUS) with MBs can be applied non-invasively after cell placement to promote extravasation and intracellular cargo release. Emerging clinical translation includes phase I trials using sonoporation to enhance gene therapy vectors in DMD and limb-girdle dystrophy (121).

Operator variability and training requirements

Despite its advantages, ultrasound-guided delivery remains operator-dependent. Studies of MSK injections report accuracy ranging from 65% to >95% depending on experience; novices achieve only 40-60% first-pass success in complex targets (122). The learning curve for proficiency (defined as >90% accuracy and <2 min procedure time) typically requires 40-80 supervised procedures for basic intra-articular injections and 100–150 for advanced perivascular or deep intramuscular regenerative delivery (123, 124).

Standardization mitigates variability. Consensus protocols now recommend specific probe frequencies, needle types, injection rates, and post-procedure imaging checkpoints. Simulation-based training with phantoms or augmented-reality needle trainers accelerates skill acquisition and reduces the number of supervised live cases needed (125). Certification pathways (e.g., RMSK and AIUM MSK Ultrasound) and proctored case logs are increasingly required for regenerative applications to ensure reproducible outcomes and patient safety.

In summary, ultrasound-guided cellular delivery has evolved from simple landmark-assisted injection to a sophisticated, multimodal platform that integrates precision targeting, real-time feedback, and biophysical enhancement. When executed with optimized technique and adequate operator training, these methods markedly improve cell retention, engraftment, and functional regeneration while maintaining an excellent safety profile. The technical principles outlined here provide the foundation for the preclinical and clinical evidence reviewed in subsequent sections.

Preclinical evidence and mechanistic insights

Preclinical studies have established proof-of-principle that ultrasound-guided cellular delivery can improve retention, engraftment, and functional regeneration compared with non-guided methods. However, the magnitude and durability of benefit vary markedly with injury model, species, and delivery strategy. This section synthesizes evidence from rodent and large-animal models, directly compares imaging-guided versus non-guided outcomes, delineates the core regenerative mechanisms, and highlights persistent translational limitations.

Animal models

Rodent muscle injury models remain the workhorse for mechanistic dissection. Cardiotoxin (CTX), barium chloride, and freeze injury produce near-complete spontaneous regeneration within 28 days with minimal fibrosis, making them ideal for isolating paracrine or fusion effects of transplanted cells (126). VML models, by contrast, recapitulate clinically relevant persistent inflammation, fibrosis, and incomplete recovery, rendering them more

sensitive to therapeutic intervention (127). In VML, MSC spheroids in fibrin-laminin hydrogels, cardiosphere-derived cells, adipose-derived stem cells in decellularized scaffolds, and iMPs have consistently increased centrally nucleated myofibers, contractile force, and reduced fibrosis (128-131). Dystrophin-deficient (mdx, mdx/scid) and collagen-VI-deficient models further demonstrate dose-dependent histological and functional rescue by iPSC-MSCs or PSC-derived progenitors, with dystrophin supplementation preventing torque decline and restoring oxidative metabolism (132-134).

Rat models (e.g., DMD rats) offer larger tissue volumes and more severe, progressive pathology closer to human disease, facilitating better engraftment studies and functional testing (135). However, xenogeneic human-cell transplantation in immunodeficient rodents yields only ~1% niche occupancy and rapid cell loss, underscoring species-specific niche incompatibility (136).

Large-animal models (porcine, ovine, and canine) better approximate human muscle size, vascular anatomy, immune responses, and biomechanical loading. Porcine VML and DMD models replicate chronic fibrosis and impaired regeneration; canine GRMD shows symptom severity closest to human Duchenne (137). Yet few studies have applied ultrasound-guided cellular delivery in these species; available data are limited to bone, ligament, or gene-delivery protocols (138). Direct comparisons of muscle regeneration across porcine, canine, and ovine models are scarce, and cost/logistical barriers restrict their use to late-stage validation. These model-specific differences in regenerative dynamics and cellular fate are summarized in Figure 4.

Murine models, while invaluable for mechanistic insights, present important limitations when extrapolating to human injuries such as VML. Mouse skeletal muscle has minimal depth, thinner fascia, and a less complex immune architecture compared with human tissue, which features greater muscle bulk, multilayered fascial planes, and more pronounced biomechanical loading. These differences can lead to overestimation of the ease and precision of ultrasound-guided injections in clinical practice, as human-scale lesions introduce challenges in injectate distribution, shear forces during delivery, and immune-mediated clearance that are underrepresented in small-animal systems. Large-animal models better approximate these complexities but remain underutilized for ultrasound-guided cellular delivery studies.

Imaging-guided vs non-guided delivery outcomes

Critical head-to-head comparisons consistently favor ultrasound-guided delivery. In rodent muscle, ultrasound guidance achieves 74-96% accuracy versus 11-72% for blind injection, with markedly improved injectate distribution and reduced leakage (21, 22). In myocardial and urethral models (relevant by analogy), ultrasound-guided or waterjet-assisted delivery yields significantly higher cell retention and functional scores than conventional needle injection (139, 140). Pulsed focused ultrasound (pFUS) combined with intravenous MSC delivery up-regulates local adhesion molecules and chemoattractants, increasing homing >2-fold and enhancing perfusion while reducing fibrosis (141). Hydrogel-supported delivery further amplifies these benefits, producing 3-45% higher donor-fiber formation and sustained ejection-fraction gains in cardiac models that translate conceptually to skeletal muscle (18).

Non-guided intramuscular injection frequently results in backflow, compartmental pooling, and rapid clearance, with <1-5% long-term engraftment in most studies (12). Although

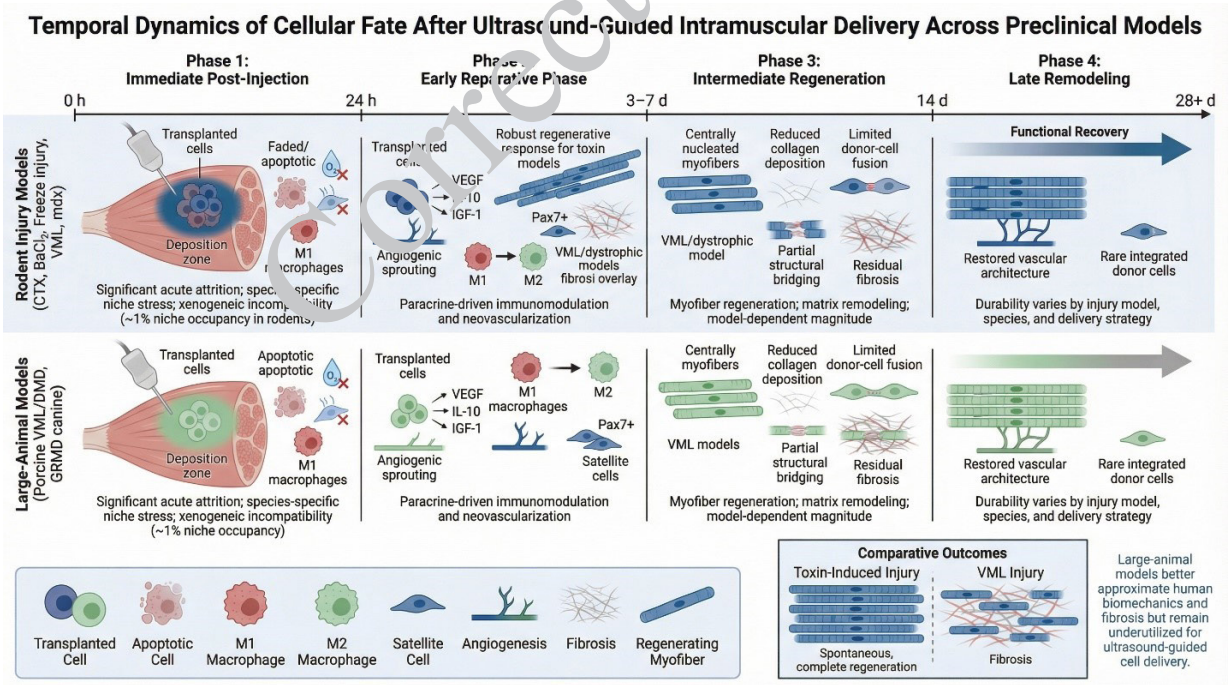

Figure 4. Temporal dynamics of cellular fate after ultrasound-guided intramuscular delivery in preclinical models
Schematic comparing rodent (CTX, $BaCl_2$, freeze, VML, and mdx) and large-animal (porcine VML and canine GRMD) muscle injury models across phases: immediate post-injection (0 hr), early reparative (24 hr to 3-7 d), intermediate regeneration (14 d), and late remodeling (28+ d). Depicts processes like cell deposition/apoptosis, M1/M2 macrophage shift, paracrine angiogenesis/immunomodulation (VEGF, IGF-1, and IL-10), Pax7+ satellite activation, myofiber regeneration, fibrosis, and recovery. Highlights model-dependent outcomes, species-specific attrition, and injury-specific endpoints (toxin vs VML)

direct quantitative retention comparisons in skeletal muscle remain limited, the available evidence indicates that real-time ultrasound visualization, hydrodissection, and fractionated injection collectively increase therapeutic cell dose at the target site by 2-5-fold, directly translating into superior histological and functional outcomes.

Mechanisms of regeneration

Engraftment is limited by hypoxia, inflammation, anoikis, and mechanical injury during injection. Prosurvival cocktails, hyaluronan-methylcellulose hydrogels, and growth-factor preconditioning (IGF-1/VEGF) increase donor-cell survival 3-fold and donor-derived fibers >45% (19). Hydrogels additionally provide a supportive niche that sustains paracrine signaling even when long-term engraftment is modest as shown in the early reparative and intermediate phases in Figure 4.

The key regenerative mechanisms of these platforms, including paracrine signaling, angiogenesis, and immunomodulation, are detailed in Section 3.1.

Key limitations of preclinical studies

Despite encouraging mechanistic insights, preclinical studies suffer from systematic weaknesses. Small sample sizes (often n=6-12 per group) inflate effect sizes and compromise reproducibility; underpowered studies are common and rarely report a priori power calculations (142). Follow-up periods are typically 2-4 weeks, missing late fibrosis, chronic inflammation, or incomplete remodeling that persists beyond 12-94 weeks in many models (143). Species differences in immune response, niche compatibility, and spontaneous regenerative capacity create large translation gaps: rodent CTX models regenerate almost completely without intervention, while human VML rarely does (12). Large-animal studies are scarce, expensive, and rarely incorporate ultrasound-guided delivery or clinically relevant endpoints. Reporting deficiencies (ARRIVE 2.0 compliance often <50%) further hinder reproducibility (144).

Collectively, these limitations explain why many promising preclinical findings have not yet translated into robust clinical benefit. The cellular-fate timeline (Figure 4) underscores that most preclinical readouts capture only the acute paracrine phase; long-term engraftment and functional integration remain the critical translational bottlenecks that ultrasound-guided delivery and advanced biomaterials must address.

Clinical applications and trials

While preclinical and technical advances have generated substantial optimism, translation into clinical practice for skeletal-muscle regeneration remains in its infancy. To date, only a handful of cell-based or cell-derived products have entered human trials, almost all in early-phase designs. The evidence base is dominated by small cohorts, heterogeneous protocols, and surrogate rather than patient-centered endpoints. This section reviews the current clinical landscape, highlights what has been achieved, and critically appraises the major barriers to definitive efficacy demonstration.

Acute muscle injuries

Sports trauma Platelet-rich plasma (PRP) remains the most frequently studied biologic in athletes, yet high-level evidence does not support accelerated return-to-play or reduced re-injury rates. A 2024 scoping review of 11 studies (four RCTs) concluded that the best-quality trials show no meaningful reduction in time-loss or recurrence, although some report short-term pain relief (6). Mesenchymal stem/stromal cells (MSCs) and their exosomes have been used off-label in professional and Olympic cohorts, but only 5.7 % of 35 published studies reached level-1 evidence, with protocols varying widely in dose, source, and timing (7). Safety appears favorable—transient fever or injection-site reactions predominate, with no serious adverse events in mid-term follow-up (145)—yet the absence of adequately powered, double-blind trials in amateur athletes precludes firm recommendations.

Ultrasound guidance markedly improves injection accuracy (74-100 % vs 11-80 % for landmark/palpation) and reduces neurovascular complications, but whether this precision translates into superior clinical outcomes remains unproven in acute muscle-strain populations (40).

Post-surgical repair Adjunctive cell therapy after surgical muscle reconstruction has been explored primarily in animal models. In rats, muscle-precursor cells combined with repair restored 102% of contralateral force versus 78% with vehicle (146). Human data are limited to one RCT of placental-derived MSCs injected during hip arthroplasty, which improved gluteus medius strength at 26 weeks (147). A meta-analysis of 44 preclinical VML studies confirmed that acellular scaffolds plus stem/progenitor cells yielded the largest functional gains (effect size 0.75)(148). Optimal timing (intra-operative vs delayed) and autologous versus allogeneic use remain unresolved; allogeneic cells trigger stronger immune responses and fibrosis in immunocompetent models (149).

Chronic conditions

Sarcopenia Two small RCTs of allogeneic or umbilical-cord MSCs in frail older adults reported modest improvements in 6-minute walk distance, short physical performance battery, and grip strength at 6 months, with dose-dependent signals (1, 2). However, a larger phase-2 trial of bimagrumab (myostatin inhibitor) increased lean mass yet failed to improve physical function beyond optimized nutrition and exercise (3). Safety concerns include potential senescent-cell transfer from aged donors and theoretical tumor-promoting effects, although serious adverse events have been rare in short-term studies (150).

Muscular dystrophies the HOPE-2 trial (n=20, late-stage Duchenne) of intravenous cardiosphere-derived cells (CAP-1002) demonstrated a clinically meaningful slowing of upper-limb deterioration at 12 months (mid-level elbow function percentile difference 36.2, P=0.014) and favorable cardiac structural changes, with an acceptable safety profile (4). A small open-label study of DT-DEC01 (myoblast fusion product) reported safety and preliminary efficacy in three patients (5). No other cell-based RCTs have been published since 2020. Systemic delivery remains inefficient in dystrophic muscle, while local ultrasound-guided approaches have not yet been tested clinically.

VML Only one prospective human series (n=13) using acellular ECM bioscaffolds plus aggressive rehabilitation reported 37% strength and 27% range-of-motion gains at 6 months (8). No cell-augmented human VML trial has been completed; early myoblast-injection studies were

safe but ineffective (151). The field therefore still relies on preclinical data showing superior outcomes when scaffolds are combined with stem/progenitor cells (148).

Clinical trial outcomes

Safety Across >900 patients in musculoskeletal MSC trials, serious adverse events are rare (incidence <1 %); transient fever, injection-site reactions, and mild hypersensitivity predominate (152). No tumorigenicity or ectopic tissue formation has been confirmed in mid-term follow-up.

Functional improvement Gains are modest and inconsistent: 2-7% improvement in 6-min walk distance or grip strength in sarcopenia trials; slowing of upper-limb decline in DMD (HOPE-2); 27-37% strength/range gains in the single VML bioscaffold series. Effect sizes rarely exceed 0.5, and placebo responses in rehabilitation arms are substantial.

Imaging outcomes Quantitative MRI (fat fraction, T2 mapping, and muscle volume) has been used in <15% of biologic trials despite high sensitivity to subclinical progression (153). When reported, fat-fraction stabilization or modest reduction correlates with functional benefit, but imaging is rarely the primary endpoint.

Critical appraisal of clinical evidence

The clinical evidence for ultrasound-guided cellular therapy in skeletal muscle, as summarized in Table 3, is characterized by four major limitations that preclude strong recommendations.

Heterogeneity dominates: cell source (autologous vs allogeneic MSCs, cardiosphere-derived cells, myoblasts), dose (10^6-10^8 cells), route (systemic vs local), timing (acute vs chronic), and co-interventions (rehabilitation protocols) vary widely even within the same indication.

Small cohorts are the rule: most studies enroll <50 patients; only HOPE-2 reached n=20 per arm. Underpowered trials inflate effect sizes and render negative results inconclusive.

Lack of standardization affects every level—cell manufacturing, potency assays, delivery technique (ultrasound vs blind), outcome selection (6MWT, grip strength, MFM-32, North Star), and imaging biomarkers. No consensus core-outcome set exists for muscle-regeneration trials.

Need for RCTs is acute. The overwhelming majority of published data derive from open-label or single-arm studies. Double-blind, placebo-controlled, adequately powered trials with predefined imaging and functional co-primary endpoints, long-term follow-up (>24 months), and cost-effectiveness analysis are required before regulatory approval or widespread adoption can be considered.

In summary, while safety appears acceptable and selected signals of biological activity exist (upper-limb preservation in DMD, modest strength gains in sarcopenia and VML bioscaffold series), the clinical evidence remains preliminary. The field now requires rigorous, standardized, multicentre RCTs that incorporate ultrasound-guided precision delivery, quantitative MRI surrogates, and patient-centered functional outcomes. Until such trials are completed, cellular therapies for skeletal-muscle regeneration should remain investigational (see Table 3 for current trial summaries).

Safety, ethical, and regulatory considerations

Despite encouraging signals of biological activity and an overall favorable short-term safety profile in early-phase trials, the path from bench to bedside for cellular therapies in skeletal muscle remains obstructed by unresolved safety uncertainties, fragmented regulatory pathways, and formidable manufacturing and reimbursement barriers. These challenges

Table 3. Summary of key clinical trials and outcomes in skeletal muscle regeneration therapies (2017-2025)

Condition	Therapy/platform	Study design/size	Key outcomes	Safety profile	References
Acute muscle injuries (sports trauma)	PRP	Scoping review: 11 studies (4 RCTs)	No reduction in return-to-play or re-injury; some short-term pain relief	Transient reactions; no serious AEs	(6)
Acute muscle injuries (athletes)	MSCs/Exosomes	Review: 35 studies (5.7% level-1 evidence)	Faster return-to-play in some series with ultrasound-guided delivery; heterogeneous protocols	Transient fever/injection-site reactions; <0.5% complications with ultrasound	(7, 40, 145)
Post-surgical repair (hip arthroplasty)	Placental-derived MSCs	RCT: NR	Improved gluteus medius strength at 26 weeks	Acceptable; no serious AEs reported	(147)
Sarcopenia	Allogeneic/UC MSCs	Two small RCTs: Frail older adults	Modest gains in 6MWD (2–7%), SPPB, grip strength at 6 months; dose-dependent	Rare senescent-cell risks; no serious AEs in short-term	(1, 2, 150)
Sarcopenia	Bimagrumab (myostatin inhibitor)	Phase-2 RCT: Older adults	Increased lean mass; no functional benefit beyond nutrition/exercise	NR; acceptable in trial	(3)
Muscular dystrophies (DMD)	CAP-1002 (Cardiosphere-derived cells, IV)	HOPE-2 RCT: n=20, late-stage	Slowed upper-limb decline (36.2% percentile difference, P=0.014); cardiac changes at 12 months	Acceptable; no serious AEs	(4)
Muscular dystrophies (DMD)	DT-DEC01 (Myoblast fusion product)	Open-label: n=3	Safety and preliminary efficacy	Safe; no AEs reported	(5)
Volumetric muscle loss (VML)	Acellular ECM bioscaffolds+rehab	Prospective series: n=13	37% strength, 27% ROM gains at 6 months	Safe; no serious AEs	(8)
VML	Myoblast injections (early studies)	NR	Safe but ineffective	Safe	(151)
Overall musculoskeletal MSC trials	MSCs	Meta-analysis: >900 patients	Serious AEs <1%; modest functional gains (effect size ≤0.5); placebo responses substantial	Transient fever/reactions predominant; no tumorigenicity in mid-term	(153)

not only slow clinical translation but also raise important ethical questions about equitable access and the responsible development of high-cost, high-risk interventions.

Safety risks

Tumorigenicity remains the most persistent theoretical concern, particularly for pluripotent-derived or extensively expanded cells. However, clinical evidence to date is reassuring: meta-analyses of >2,600 patients receiving intravascular MSCs found no increased malignancy risk (RR 0.93, 95% CI 0.60–1.45)(154), and long-term follow-up of spinal-cord-injury MSC trials (n=670) reported zero tumor-related serious adverse events (155). Local intramuscular delivery further reduces systemic dissemination risk compared with intravenous administration, with preclinical biodistribution studies showing negligible off-target accumulation after IM injection (156). Nonetheless, donor-derived senescence, epigenetic drift during expansion, and rare cases of malignant transformation in heavily manipulated iPSC lines continue to necessitate rigorous karyotyping, whole-genome sequencing, and extended tumorigenicity assays prior to clinical use (157).

Immune reactions constitute the second major risk axis. Allogeneic MSCs trigger donor-specific antibodies in 11-15% of recipients, with repeated dosing amplifying humoral and cellular responses (158). In immunocompetent animal models, allogeneic cells induce stronger fibrosis and poorer regeneration than autologous or syngeneic counterparts (149). Clinical translation has been hampered by this immunogenicity; HLA gene-editing strategies (CRISPR-mediated HLA-A/B knockout) have shown >90% reduction in T-cell recognition in preclinical models but have not yet entered muscle-specific trials (159). Autologous or iPSC-derived approaches avoid allo-rejection but carry higher manufacturing complexity and cost.

Infection and procedural complications are rare when GMP standards and image guidance are employed. Large registries of ultrasound-guided musculoskeletal injections report infection rates <0.015-0.1%, with 99% of complications minor (160). Cryopreservation–thaw cycles can reduce microbial viability but also cause 10–30% loss of cell potency, underscoring the need for optimized closed-system processing (161). Overall, serious procedure-related infections remain exceptional in properly executed trials.

Regulatory landscape

The regulatory environment for muscle-directed cellular therapies is shaped by the Advanced Therapy Medicinal Product (ATMP) framework in Europe and the Regenerative Medicine Advanced Therapy (RMAT) designation in the United States.

The FDA classifies most cell-based muscle products as biologics requiring BLA approval. RMAT designation offers early interaction, rolling review, and surrogate-endpoint flexibility, yet real-world data show RMAT products paradoxically experience longer development timelines than non-designated therapies, and approval rates remain low (162). The EMA classifies these products as ATMPs under Regulation (EC) No 1394/2007, with the Committee for Advanced Therapies (CAT) providing scientific advice and the PRIME scheme accelerating assessment for unmet needs. Both agencies demand comparable demonstration of safety, purity, potency, and efficacy, yet EMA more frequently grants conditional marketing authorization and imposes additional post-authorization safety studies (163).

Harmonisation remains incomplete. Divergent requirements for potency assays, long-term follow-up duration, and acceptance of real-world evidence create parallel development pathways that increase cost and delay global access (164). Japan's Sakigake and conditional/time-limited approval pathways have been the most permissive, but post-marketing safety obligations are correspondingly strict.

Manufacturing and cost barriers

GMP cell production is the single largest translational bottleneck. Allogeneic "off-the-shelf" products require master/working cell banks, extensive adventitious-agent testing, and lot-release assays that can consume >50% of total development cost (165). Donor-to-donor variability in proliferation, immunomodulatory potency, and senescence further complicates scale-up and comparability (166).

Scalability is constrained by bioreactor geometry, shear sensitivity of anchorage-dependent cells, and downstream processing yields. Current automated platforms (e.g., CliniMACS Prodigy and Cocoon) can reach 10^9–10^10 cells per batch, yet commercial allogeneic muscle indications may require 10^11–10^12 cells per dose at acceptable cost-of-goods (<US$50,000–100,000)(167). Decentralized point-of-care manufacturing offers a potential solution but introduces new regulatory and quality-control complexities.

Reimbursement issues are acute. One-time administration of high-cost biologics strains traditional fee-for-service models. Innovative payment schemes—annuity models, outcome-based rebates, and managed-entry agreements—are being piloted in Europe more aggressively than in the ultrasound, yet payers in both regions demand robust long-term evidence that current early-phase trials cannot yet provide (168). In low- and middle-income countries the gap is even wider, with almost no regulatory frameworks or reimbursement pathways for ATMPs (169).

In summary, while the safety database is accumulating favorably and regulatory tools exist to accelerate promising candidates, the combination of immunogenicity risks, manufacturing complexity, and reimbursement uncertainty continues to limit broad clinical adoption. Addressing these interconnected challenges through harmonized international standards, automated scalable platforms, and innovative payment models will be essential for realizing the therapeutic promise of ultrasound-guided cellular therapies in skeletal muscle.

Translational challenges and future directions

The preceding sections have demonstrated that ultrasound-guided cellular delivery holds genuine promise for skeletal muscle regeneration, yet the gap between preclinical efficacy and clinical impact remains wide. Realizing this potential will require concerted efforts to standardize protocols, harness artificial intelligence, personalize cell products, integrate combination strategies, and establish a clear translational roadmap. This final section outlines these priorities and proposes a realistic timeline for clinical adoption between 2025 and 2040.

Standardization of protocols

Standardized protocols for ultrasound-guided cellular therapy are currently absent, leading to marked heterogeneity

in cell preparation, delivery technique, dosing, and outcome reporting across trials. Multicentre studies implementing harmonized MSC culture procedures still report slight quantitative differences attributable to laboratory-specific practices, underscoring that harmonization reduces but does not eliminate inter-operator and inter-site variability (170). Ultrasound parameters exert non-linear effects on stem-cell viability and differentiation: low-intensity pulsed ultrasound (1-3 MHz, <500 mW/cm²) promotes differentiation, while deviations outside narrow windows can diminish efficacy or induce unintended stress responses (171). Without minimal reporting standards for frequency, intensity, duty cycle, and exposure duration, reproducibility suffers (172).

Operator dependency remains a critical bottleneck. Intralesional and perivascular micro-injections are highly technical, specialized skills with a steep learning curve. Studies of MSK injections report accuracy ranging from 65% to >95% depending on experience; novices achieve only 40-60% first-pass success in complex targets (122). The learning curve for proficiency (defined as >90% accuracy and <2 min procedure time) typically requires 100-150 supervised procedures for advanced perivascular or deep intramuscular regenerative delivery (123, 124). Formal credentialing in musculoskeletal US (e.g., RMSK or AIUM certification), simulation-based training with phantoms or augmented-reality needle trainers, and proctored case logs are therefore essential to ensure safety, consistency, and reproducible outcomes across centers.

Expert groups have proposed consensus frameworks addressing GMP manufacturing, potency assays, release criteria, and quality-control checkpoints, yet adoption remains inconsistent (173, 174). A CLINIC-STRA-SVF guideline (33 items) for stromal vascular fraction studies and extensions of CONSORT for cell-therapy trials represent important steps, but compliance is suboptimal even in high-impact journals (175). Centralized protocol repositories, mandatory training curricula, and governance structures with real-time quality monitoring are urgently needed to enable multicentre comparability and regulatory acceptance.

AI-guided ultrasound navigation

Artificial intelligence is poised to transform ultrasound-guided delivery from an operator-dependent art into a reproducible, high-precision procedure. Deep-learning models (CNNs, R-CNNs) combined with statistical filtering already achieve sub-millimetre needle-tip localization (0.48-0.74 mm error) and >99% precision/recall at real-time frame rates (176, 177). In prospective musculoskeletal injection studies, AI assistance improved anatomical structure identification (88.8% vs 77.4%), reduced needle passes, shortened procedure time, and enhanced pain relief and range-of-motion outcomes compared with manual guidance (45).

For regenerative applications, AI can automate lesion segmentation, optimize needle trajectory to vascular niches or fibrotic zones, and provide immediate feedback on injectate distribution. Challenges include scarcity of annotated ultrasound datasets for regenerative procedures and the need for domain adaptation across different machines and patient habitus. Prospective validation in cell-therapy trials is the next critical step.

Personalized cell engineering

Patient-specific iMPs and CRISPR-edited MSCs offer the prospect of autologous, hypoimmunogenic, or enhanced-potency products. CRISPR-mediated HLA-A/B knockout reduces T-cell alloreactivity >90 % in preclinical models while preserving engraftment (159). Targeted insertion of survival factors (IGF-1, VEGF), anti-fibrotic miRNAs, or myogenic master regulators can further tailor cells to individual disease microenvironments. Omics profiling (single-cell RNA-seq, miRNA panels) already identifies responder versus non-responder MSC signatures in osteoarthritis, enabling pre-treatment stratification (178).

However, manufacturing complexity, cost, and unresolved tumorigenicity risks from reprogramming or off-target editing currently confine these approaches to academic proof-of-concept studies. Scalable, closed-system, automated platforms with integrated potency and genomic-safety assays are prerequisites for personalized therapies to reach routine clinical use.

Combination therapies

Single-modality interventions have reached diminishing returns. Synergistic combinations are now essential.

Gene therapy+cells: CRISPR-corrected autologous iPSC-derived progenitors or lentivirally enhanced MSCs (e.g., overexpressing dystrophin mini-genes or anti-inflammatory cytokines) have shown superior engraftment and functional rescue in DMD models compared with unmodified cells. Early human data are emerging from *ex vivo* gene-edited HSC therapies in blood disorders; analogous muscle-directed strategies are in late preclinical development.

Biomaterials+ultrasound: Injectable hydrogels or decellularized ECM scaffolds protect cells from injection shear, provide sustained paracrine signaling, and improve retention 5-12-fold (264). When combined with sonoporation or ultrasound-targeted microbubble destruction, these constructs enable on-demand release of exosomes or growth factors, further enhancing angiogenesis and myogenesis while reducing fibrosis (179).

Roadmap to clinical adoption (2025-2040)

A realistic, milestone-driven roadmap is outlined in Figure 5.

- 2025-2028 (Phase II optimization): Standardized ultrasound-guided delivery protocols, AI navigation validation, first-in-human combination trials (MSC+sonoporation or hydrogel), and establishment of core outcome sets (functional + quantitative MRI).
- 2028-2032 (Pivotal Phase III): Multicentre RCTs in VML, sarcopenia, and DMD using autologous or hypoimmunogenic allogeneic products; demonstration of cost-of-goods <US$80,000 and durable functional benefit (>24 months).
- 2032-2036 (Regulatory & reimbursement): First approvals via RMAT/ATMP/PRIME pathways; value-based payment models (annuity, outcomes-linked rebates); decentralized manufacturing hubs in major trauma centers.
- 2036-2040 (Broad adoption): Personalized iPSC-MSC therapies for rare dystrophies; point-of-care sonoporation-enhanced exosome delivery for acute sports injuries; integration into standard rehabilitation protocols for sarcopenia and post-traumatic VML.

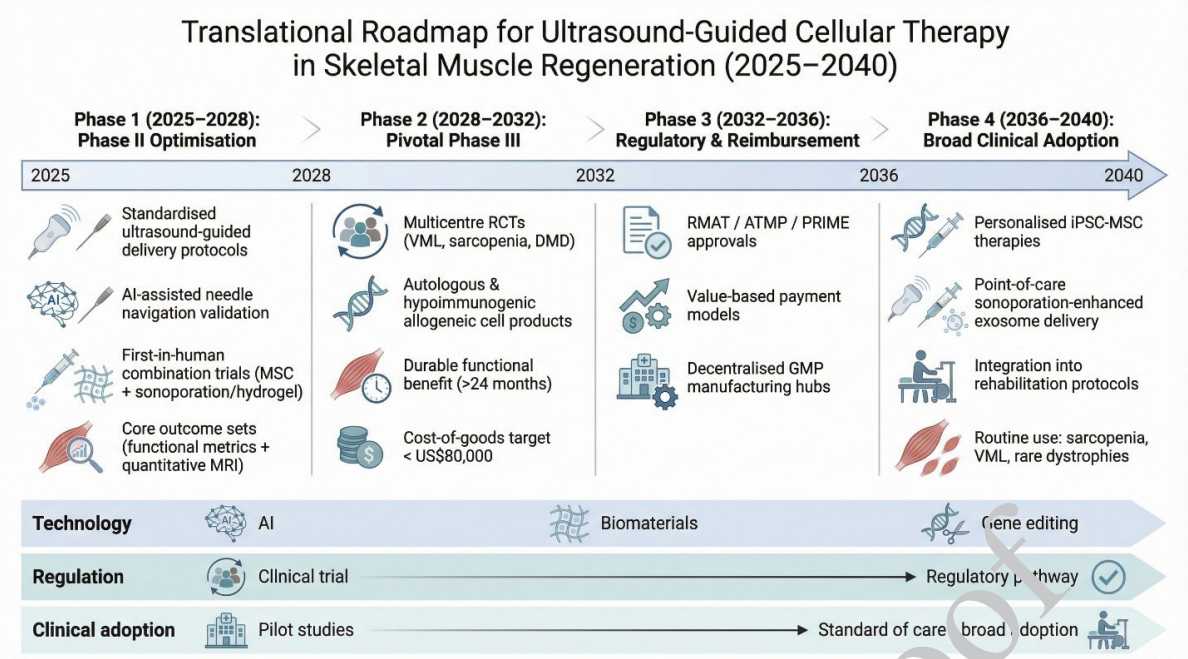

Figure 5. Translational Roadmap for Ultrasound-Guided Cellular Therapy in Skeletal Muscle Regeneration (2025–2040)
This timeline outlines key phases from optimization and pivotal trials to regulatory approval and broad clinical adoption, incorporating advancements in AI, biomaterials, and gene editing. Milestones include standardized protocols, multicenter RCTs, RMAT/ATMP/PRIME approvals, and personalized iPSC-MSC therapies, with a focus on cost-effectiveness and integration into routine care for conditions like VML, sarcopenia, and DMD

While this timeline is optimistic, it acknowledges the inherent risks and uncertainties inherent in clinical translation, including Phase III trial success rates, regulatory harmonization challenges, and evolving reimbursement landscapes. This phased approach is visualized in Figure 5.

Achieving this vision will require sustained public-private partnership, international harmonization of potency and safety assays, and rigorous health-economic modelling. If these challenges are met, ultrasound-guided cellular therapies have the potential to transform the management of one of the most common and functionally devastating tissue injuries worldwide.

Conclusion

Ultrasound-guided cellular therapies represent a synergistic convergence of biological insights and imaging precision, poised to address the regenerative failures inherent in skeletal muscle injuries and degenerative conditions. As delineated throughout this review, native healing cascades—encompassing inflammation resolution via M1-to-M2 macrophage transitions, satellite cell (MuSC) activation through Notch/PI3K/mTOR pathways, and remodeling supported by FAPs and angiogenesis—frequently falter in severe, chronic, or aged contexts, leading to fibrosis, vascular insufficiency, and functional deficits (2.1 Native Muscle Healing Cascade, 2.2 Why Regeneration Fails). Cellular platforms, including MSCs, MuSCs, iPSC-derived progenitors, and exosomes, counter these through paracrine (IGF-1/VEGF/miR-494), immunomodulatory (IL-10 up-regulation, Treg expansion), and differentiative mechanisms, achieving 2-5-fold retention and recovery gains when delivered with biomaterials or sonoporation (3.1 Stem and Progenitor Cell Types, 3.2 Cell-Free Approaches, 6.3 Mechanisms of Regeneration). Musculoskeletal US enables this by providing real-time lesion mapping, perivascular targeting, injectate tracking, and biomarkers like SWE (correlating r=0.68-0.87 with fibrosis)(4.1 Ultrasound Physics Relevant to Regenerative Delivery, 4.3 Advanced Ultrasound Technologies). Preclinical evidence consistently favors guided over blind delivery (74-96% accuracy vs 11-72%), with large-animal models underscoring scalability challenges (6.1 Animal Models, 6.2 Imaging-Guided vs Non-Guided Delivery Outcomes). Clinically, early-phase trials demonstrate safety (serious adverse events <1%) and modest benefits—e.g., slowed DMD progression in HOPE-2, 37% strength gains in VML bioscaffolds—but heterogeneity and underpowering limit generalizability (7.1 Acute Muscle Injuries, 7.2 Chronic Conditions, 7.3 Clinical Trial Outcomes). Regulatory and manufacturing barriers, including immunogenicity (11-15% donor-specific antibodies) and high costs (>50% development budget), further impede translation, though hypoimmunogenic editing and automated platforms offer solutions (8.1 Safety Risks, 8.3 Manufacturing and Cost Barriers).

The clinical relevance of this integration is profound: for athletes with acute strains, ultrasound-guided exosomes could accelerate return-to-play beyond PRP's inconsistent outcomes; in sarcopenia, allogeneic MSCs might enhance grip strength and mobility, addressing frailty's economic burden; and for dystrophies like DMD, sonoporation-enhanced gene-corrected progenitors could preserve function long-term (7.4 Critical Appraisal of Clinical Evidence, 9.4 Combination Therapies). Critically, AI navigation and standardized protocols mitigate operator variability (learning curve 100-150 procedures), while combinations (cells+hydrogels+focused ultrasound) amplify engraftment and paracrine effects, potentially shifting from modest (effect size 0.5) to transformative gains (5.4 Operator Variability and Training Requirements, 9.1 Standardization of Protocols, 9.2 AI-Guided Ultrasound

Navigation).

Research priorities must focus on multicenter RCTs with core outcome sets (quantitative MRI, patient-centered endpoints like 6-minute walk), long-term follow-up (>24 months), and health-economic analyses to demonstrate value (9.5 Roadmap to Clinical Adoption). Addressing gaps—small cohorts, species differences, and reimbursement models (e.g., outcome-based rebates)—requires public–private partnerships for scalable GMP production and harmonized ATMP/RMAT pathways (8.2 Regulatory Landscape, 9.3 Personalized Cell Engineering). Preclinical underpowering (n=6-12) and short timelines (2-4 weeks) inflate effects; future studies should prioritize large-animal validation and ARRIVE-compliant reporting (6.4 Key Limitations of Preclinical Studies).

Ultimately, when milestones like AI-optimized delivery (2025-2028), pivotal trials (2028-2032), and personalized products (2036-2040) are achieved, ultrasound-guided cellular therapies will transform skeletal muscle regeneration from investigational to standard-of-care, offering durable solutions where endogenous repair fails and current treatments fall short.

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Authors' Contributions

C Y, X L, W Z, and L Z wrote and finalized the draft. All authors wrote and finalized the investigation.

Conflicts of Interest

There are no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

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