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REVIEW ARTICLE

Bioprinting strategies for skeletal muscle regeneration: Advances in bioinks, technologies, and functional reconstruction

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Abstract

Volumetric muscle loss (VML) presents a significant clinical challenge because the intrinsic regenerative capacity of skeletal muscle is insufficient to repair extensive defects, and current therapeutic strategies remain inadequate. Bioprinting has emerged as a transformative approach, enabling the spatially controlled deposition of cells, biomaterials, and biochemical cues to create functional, biomimetic muscle tissues. This review offers a comprehensive overview of recent advancements in bioink development, bioprinting technologies, and functional reconstruction strategies for skeletal muscle regeneration. Bioinks derived from natural, synthetic, and composite materials are examined in terms of their effectiveness in supporting myogenesis, promoting cellular alignment, and facilitating neurovascular integration. We compare key bioprinting techniques—including extrusion-based, inkjet, and laser-assisted printing—highlighting their respective strengths and limitations in achieving structural fidelity and multicellular complexity. Emerging technologies such as coaxial and microfluidic-assisted printing are also discussed for their potential to fabricate aligned, anisotropic muscle constructs with hierarchical architectures. Functional outcomes are synthesized from *in vitro* assays (e.g., contractility, gene expression) and *in vivo* studies using VML models, with a focus on vascularization, innervation, and force restoration. Despite significant progress, substantial challenges remain in achieving complete neurovascular integration, long-term functionality, and clinical scalability. Moving forward, future efforts should emphasize the development of dynamic, bioresponsive materials, integration with electrical and mechanical stimulation, and the establishment of standardized preclinical protocols. By bridging material innovation, structural design, and biological functionality, bioprinting holds great promise for next-generation, clinically relevant skeletal muscle regeneration.

Keywords: 3D bioprinting; Hydrogel bioinks; Muscle tissue engineering; Myogenesis; Regenerative medicine

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1. Introduction

Skeletal muscle is a highly regenerative tissue that relies on tissue-resident stem cells to repair damage after injury.[1–3] However, its intrinsic self-repair capacity becomes insufficient in the case of extensive damage, as observed in volumetric muscle loss (VML), which involves the loss of more than 20% of muscle mass.[4,5] Skeletal muscle injuries, especially VML, therefore represent a significant clinical challenge due to the limited regenerative capacity of adult muscle tissue when large-scale defects occur.[6,7] These injuries can result from trauma, tumor resection, congenital abnormalities, or degenerative diseases such as muscular dystrophies.[8–10] Traditional treatment approaches—including autologous muscle grafts, physical therapy, and pharmacological interventions—often lead to suboptimal outcomes, such as fibrosis, incomplete functional recovery, and donor site complications.[11–15] Therefore, there is a pressing need for bioengineered solutions that can restore both the structure and function of damaged skeletal muscle.

Bioprinting has emerged as a powerful technology in regenerative medicine by enabling the precise spatial deposition of cells, biomaterials, and biochemical signals to fabricate three-dimensional (3D), biomimetic tissue constructs.[16–18] In the context of skeletal muscle, successful regeneration requires not only the recreation of aligned, contractile myofibers but also the establishment of vascular and neural networks to ensure tissue viability and physiological functionality.[19–24]

However, despite growing interest and technological progress, several critical gaps remain unaddressed. Many existing studies report limited long-term contractile function, insufficient neurovascular integration, and poor scalability of printed constructs.[25–29] The lack of standardized bioink formulations that simultaneously support mechanical integrity, myogenic differentiation, and cellular viability also hinders reproducibility and clinical translation.[30,31] Moreover, functional evaluation metrics vary widely across studies, making cross-comparison and validation challenging.[32,33]

To address these limitations, this review offers a comprehensive and structured synthesis of recent advances in bioprinting strategies for skeletal muscle regeneration, with a unique focus on three interdependent pillars: (i) bioink development, (ii) bioprinting technologies, and (iii) functional reconstruction. Unlike previous reviews that emphasize materials or methods in isolation, this work integrates biological requirements, engineering innovations, and translational benchmarks into a cohesive framework. Finally, we propose future directions and design principles for developing next-generation muscle constructs with clinically relevant performance.

2. Methodology

This systematic review was conducted using a transparent, rigorous, and reproducible approach to ensure comprehensive coverage of recent studies on bioprinting strategies for skeletal muscle regeneration.

Relevant literature was retrieved from five major electronic databases: PubMed, Web of Science, Scopus, Embase, and Google Scholar, covering the period from January 1, 2020, to May 31, 2025. The search strategy combined Medical Subject Headings (MeSH) and free-text keywords related to bioprinting, skeletal muscle, regeneration, VML, bioinks, angiogenesis, and myogenesis. Where possible, searches were restricted to titles and abstracts, and only peer-reviewed, English-language publications were included.

The initial search yielded 528 records, distributed as follows: PubMed (n = 94), Web of Science (n = 82), Scopus (n = 88), Embase (n = 96), and Google Scholar (n = 168). After removing 128 duplicates, 400 unique articles remained for screening.

The inclusion criteria were as follows: (i) written in English and published in peer-reviewed journals; (ii) described original experimental studies or systematic/comprehensive reviews; (iii) focused explicitly on bioprinting approaches for skeletal muscle regeneration; and (iv) reported functional evaluations, either *in vitro* (e.g., contractility, myogenic differentiation markers) or *in vivo* (e.g., force generation, vascular integration). Studies were excluded if they: (i) did not pertain specifically to skeletal muscle (n = 38); (ii) lacked functional assessment of the bioprinted constructs (n = 50); (iii) did not involve bioprinting-specific methodologies (n = 37); or (iv) were not peer-reviewed, including conference abstracts and preprints (n = 31).

All articles were screened independently by two reviewers in a three-stage process: title, abstract, and full-text review. Any disagreements were resolved through discussion or adjudication by a third reviewer. The entire review process adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. The detailed selection process is summarized in Figure 1.

To enable transparent cross-study comparisons, we systematically extracted and organized core methodological parameters from each included study into Table S1. Specifically, five key items were documented per study: (i) experimental design (e.g., rat VML model, *in vitro* aligned 3D constructs, neuromuscular junction models);

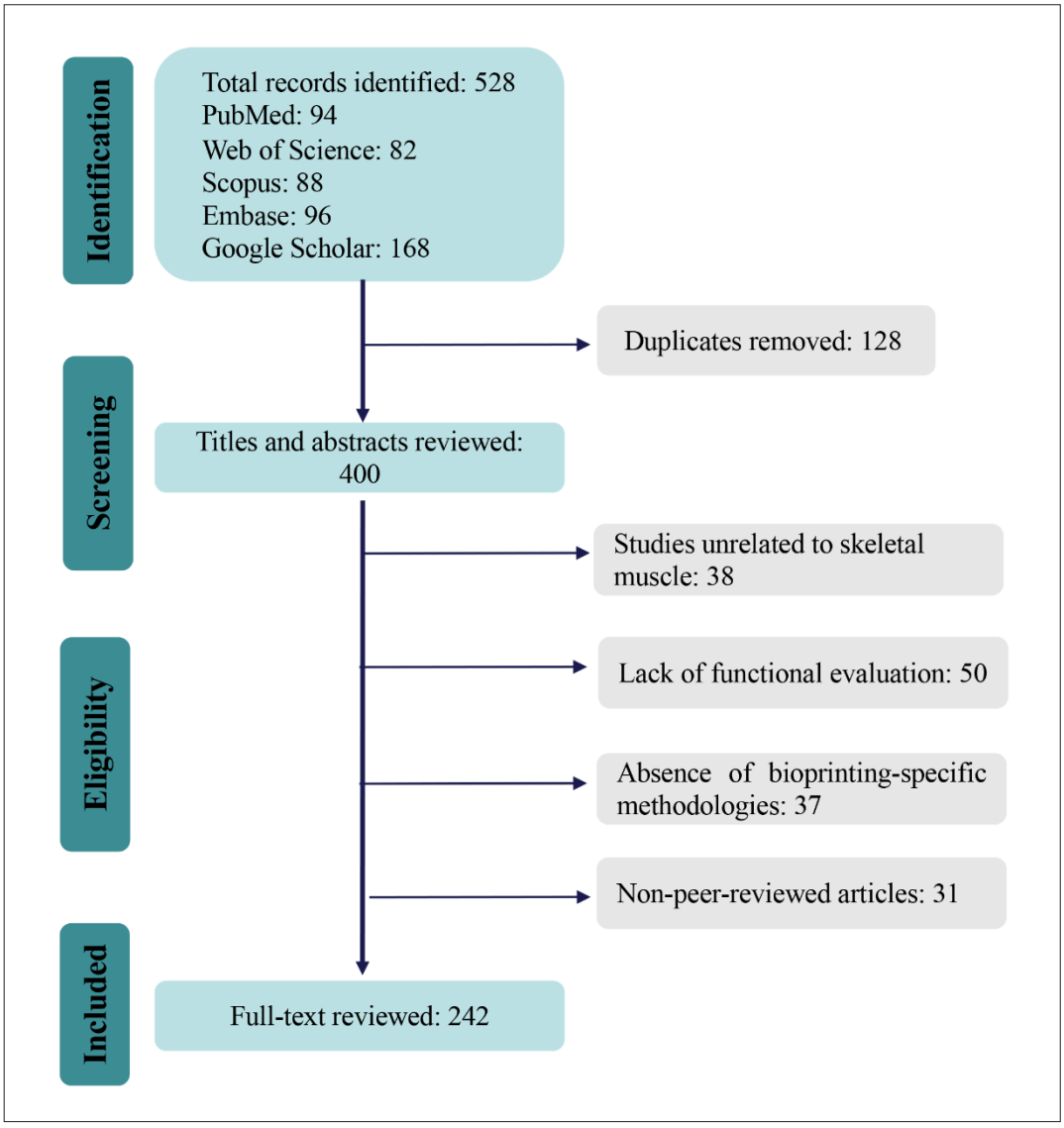

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies.

(ii) primary scaffold or bioink formulation; (iii) species or cell line used; (iv) reported outcome metrics such as tetanic force, viability, vascularization, calcium transients, or neuromuscular junction formation; and (v) a reference ID (Ref_No) corresponding to our main reference list. Among the included studies, collagen/ extracellular matrix (ECM)-based hydrogels were the most common materials (33%), followed by gelatin methacrylate (GelMA) composites (21%). Rodent models remained the dominant *in vivo* systems, with rats and mice representing 28% and 17% of studies, respectively, while 26% employed human-derived cells for *in vitro* applications. Functional evaluations were distributed across five principal readouts: contractile force (46%), viability or metabolic activity (38%), vascularization (24%), electrophysiology or calcium imaging (19%), and neuromuscular junction formation (12%). This structured extraction enabled consistent cross-study synthesis and highlighted current trends in the design and evaluation of bioprinted skeletal muscle constructs.

3. Structural and biological foundations of skeletal muscle regeneration

Skeletal muscle exhibits a highly organized and hierarchical structure that is essential for its contractile and metabolic functions.[34,35] It consists of parallel arrays of multinucleated myofibers, embedded within an ECM and

enclosed by connective tissue layers.[36] This architecture enables directional force transmission and mechanical stability.[37,38] Surrounding the muscle fibers is a dense network of blood vessels and peripheral nerves, which are critical for oxygen and nutrient delivery, as well as neuromuscular coordination.[39–41] The anisotropic and compartmentalized nature of skeletal muscle presents unique design requirements that must be recapitulated in tissue-engineered constructs.[42]

Endogenous skeletal muscle regeneration is a tightly regulated, multistep process involving both resident and recruited cell populations.[13,43,44] Upon injury, an initial inflammatory phase activates quiescent satellite cells—the primary muscle stem cells—which then proliferate, differentiate, and fuse to form new myotubes.[1,3,45–47] Concurrently, macrophages coordinate tissue clearance and remodeling, while vascular endothelial cells initiate angiogenic processes to re-establish perfusion.[43,44,48–50] Motor neurons gradually reconnect with regenerating fibers to restore neuromuscular junctions and functional innervation.[51,52] This dynamic interplay among myogenic, vascular, and neural elements underscores the integrated nature of functional muscle repair (Figure 2).

4. Engineering strategies: design principles and bioink optimization

4.1. Design requirements for functional muscle constructs

The complexity of skeletal muscle regeneration defines a rigorous set of criteria for tissue engineering.[53,54] Foremost

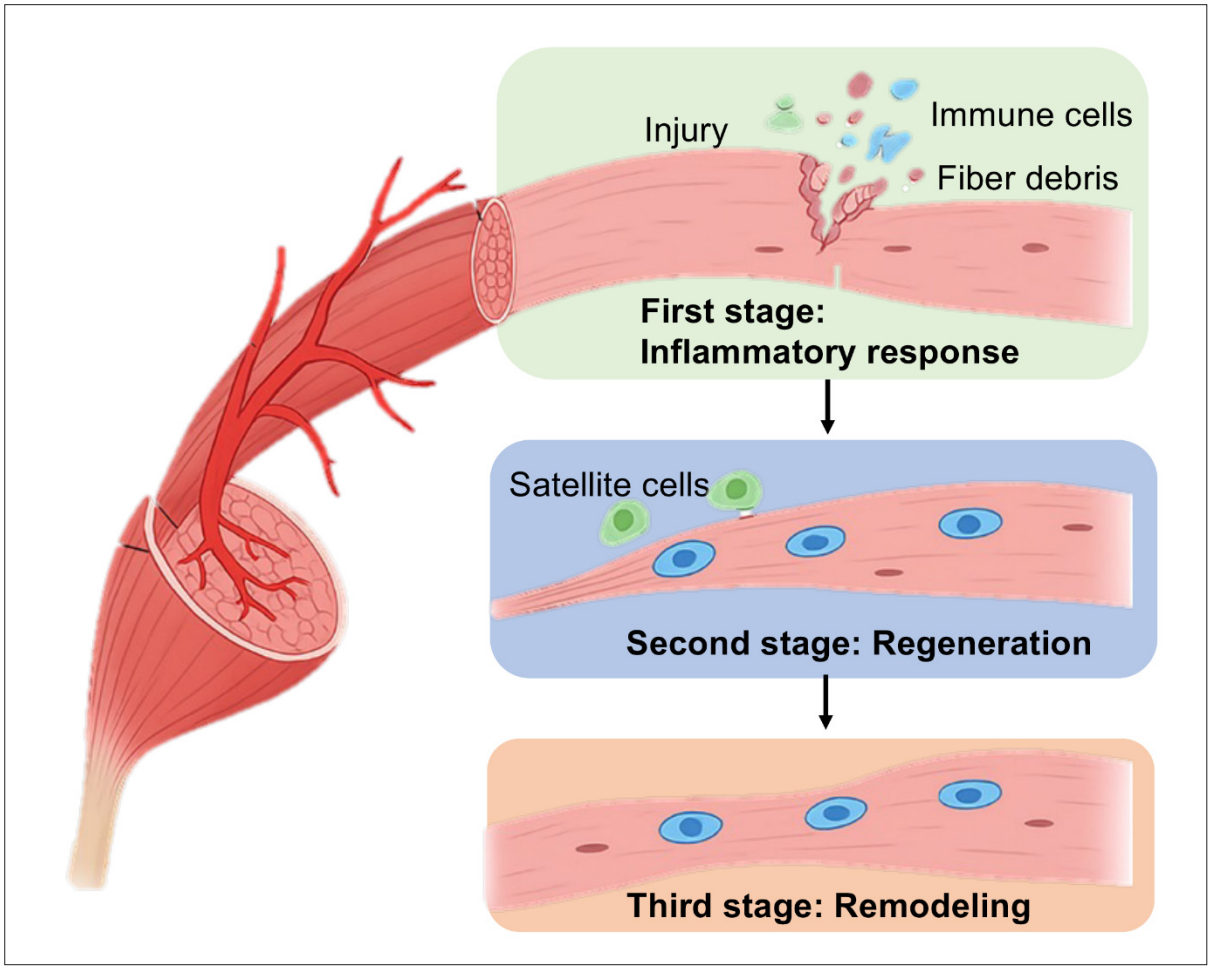

Figure 2. Schematic illustration of native skeletal muscle structure and regenerative phases. This figure depicts the multistep process of skeletal muscle regeneration following injury. On the left, the architecture of native skeletal muscle is shown, including aligned muscle fibers, motor neurons, and surrounding capillaries. The first stage illustrates the inflammatory response, with immune cells clearing debris from damaged fibers. The second stage represents satellite cell activation, proliferation, and differentiation into new myogenic cells. The third stage demonstrates tissue remodeling and regeneration, during which new muscle fibers mature and re-establish functional architecture. This integrated process highlights the dynamic interplay between myogenic, vascular, and neural components essential for muscle repair.

is the recreation of aligned, contractile myofibers capable of generating directional force, necessitating control over cellular orientation and myotube maturation.[55] Equally important is the establishment of a perfusable vascular network to ensure long-term tissue viability, particularly in constructs exceeding the diffusion limit.[56,57] Reinnervation is essential for restoring physiological control, as neuromuscular junctions enable stimulus-responsive contraction.[58,59] In addition, mechanical properties must approximate native muscle elasticity to support load-bearing functionality.[60,61] These multidimensional requirements highlight the need for integrative approaches that combine biological fidelity with structural and mechanical optimization.

The complex architecture and function of skeletal muscle demand bioprinting strategies with high spatial and compositional precision.[19,62] Conventional scaffold-based approaches fail to replicate the anisotropic structure and cellular heterogeneity of native muscle.[63,64] In contrast, bioprinting enables the fabrication of constructs with programmable architecture, controlled cell alignment, and multi-lineage distribution.[65]

To fulfill biological requirements, bioinks must: (i) support myogenic differentiation; (ii) promote cellular alignment; (iii) facilitate vascularization and innervation; and (iv) maintain print fidelity and mechanical integrity.[19,21,23,66–70] Emerging techniques such as coaxial extrusion and microfluidic-assisted printing offer new avenues for producing biomimetic muscle tissues with improved structural and functional fidelity.[71,72] These considerations inform the rational design of next-generation bioprinted muscle constructs, as elaborated in the following sections.

4.2. Bioink formulation and material strategies

The development of bioinks is central to advancing skeletal muscle bioprinting, as it directly influences printability, biocompatibility, cellular behavior, and tissue maturation.[66,73] Muscle-specific bioinks must achieve not only high-resolution patterning but also recreate the biochemical and mechanical cues essential for myogenesis, vascularization, and innervation.[19,23,74,75] Recent studies have focused on tailoring bioink formulations through variations in material origin, tuning of mechanical properties, incorporation of biological factors, and the integration of functional enhancers to improve construct performance.[76,77]

Natural biomaterials such as GelMA, collagen, fibrin, and decellularized extracellular matrix (dECM) have been widely used due to their intrinsic bioactivity and ability to support myogenic differentiation.[78–82] GelMA, for instance, offers tunable stiffness and excellent printability, while retaining bioactive motifs that promote myoblast adhesion and proliferation.[83] Collagen and fibrin closely mimic the native ECM, supporting myotube formation and initial alignment.[84]

Decellularized skeletal muscle-derived ECM provides an even more tissue-specific biochemical milieu, including preserved muscle-specific growth factors and ECM proteins.[85] However, these materials often suffer from low mechanical integrity, rapid degradation, and batch-to-batch variability, limiting their applicability in load-bearing muscle constructs.[86]

Regulatory and manufacturing hurdles remain significant for dECM-based bioinks. Because dECM is procured from animal or human tissues, each batch carries inherent donor-to-donor variability in biochemical composition, residual DNA, and mechanical properties, which can translate into inconsistent cell-fate outcomes after printing.[87] Lot-to-lot testing, therefore, must extend beyond sterility and endotoxin assays to include quantitative proteomics, glycosaminoglycan content, and rheological fingerprints.[88] From a regulatory standpoint, the United States Food and Drug Administration (FDA) typically considers cell-laden dECM constructs as combination products and applies both medical device (21 CFR 820) and biologics (21 CFR 1271) requirements, whereas the European Medicines Agency (EMA) classifies them as advanced-therapy medicinal products (ATMPs) that must satisfy Good Manufacturing Practice (GMP) Annex 1 and ISO 22442 for animal-derived materials. These frameworks mandate traceable sourcing, validated decellularization/viral inactivation, and release criteria that demonstrate batch equivalence in safety, sterility, and functional potency. Meeting such standards at scale remains challenging, particularly when kilogram-scale dECM is required for large human grafts. Emerging solutions include: (i) pooled donor lots combined with statistical process control; (ii) in-line Raman or Fourier transform infrared spectroscopy for real-time compositional monitoring; and (iii) hybrid bioinks that blend dECM with synthetic polymers to buffer mechanical variability while retaining bioactivity.[89]

Synthetic polymers such as polycaprolactone (PCL), polyethylene glycol diacrylate (PEGDA), and polylactic acid offer superior mechanical properties and slower degradation kinetics.[90–92] These materials can provide structural scaffolding for large-volume muscle defects and allow for complex construct shaping during printing.[93]

Nevertheless, synthetic bioinks are generally biologically inert, requiring surface modification (e.g., RGD peptide grafting, plasma treatment) or blending with bioactive components to promote cell adhesion and

differentiation.[94–97] Furthermore, their high stiffness may hinder myotube elongation and reduce cell viability unless carefully tuned.[67]

To overcome the inherent limitations of single-component bioinks, composite systems that integrate both natural and synthetic polymers have been extensively developed.[97–101] These formulations combine the bioactivity of natural materials with the mechanical strength of synthetic polymers, facilitating improved structural integrity while maintaining cellular compatibility and functionality.[101–103] Representative combinations, such as GelMA with PCL or fibrin with PCL, demonstrate enhanced stability under physiological conditions alongside support for tissue-specific cellular processes.[104–107] Similarly, PEGDA blended with collagen improves shape fidelity without sacrificing biocompatibility.[108,109]

These composite systems can be further optimized through tailored crosslinking methods, tunable stiffness, and spatial gradient structuring to address region-specific functional demands within engineered muscle tissue.[110,111] Softer matrix regions may be designed to promote myofiber alignment and maturation, while stiffer domains can serve as anchorage sites or load-bearing compartments, thereby enabling the construction of physiologically relevant muscle architectures.[112]

4.3. Smart bioinks and translational considerations

Incorporating bioactive molecules into bioinks has emerged as a key strategy to enhance muscle regeneration.[113,114] For instance, insulin-like growth factor-1 (IGF-1) accelerates myogenic differentiation, vascular endothelial growth factor promotes angiogenesis, and nerve growth factor supports reinnervation.[115–118] Simultaneously, nanomaterials such as gold nanoparticles, graphene oxide, and carbon nanotubes have been explored to improve electrical conductivity, cellular alignment, and mechanical reinforcement.[119–124] These additives can facilitate neuromuscular signal transmission and promote the maturation of engineered myotubes.[125,126] More recently, electrically conductive hydrogels incorporating materials such as polypyrrole or poly(3,4-ethylenedioxyt hiophene):polystyrene sulfonate have shown promise in enabling stimulus-responsive behavior, which is essential for restoring muscle contractile function.[127,128]

The field is witnessing a shift toward 4D bioinks, which undergo dynamic changes in response to stimuli such as temperature, pH, enzymes, or electrical input.[80,129,130] Such smart bioinks can simulate the adaptive behavior of native muscle, enabling constructs to respond to mechanical loading or neural cues in a physiologically relevant manner.[19,131,132] Additionally, advances in microfluidic-based mixing, multi-phase emulsions, and shear-thinning formulations are enabling the generation of heterogeneous bioinks capable of spatial patterning, gradient generation, and multi-cell co-printing—crucial for recapitulating the compartmentalized structure of skeletal muscle.[71,133–137] A comparative summary of representative bioinks, along with their advantages and limitations, is provided in Table 1.

While substantial progress has been made in developing muscle-specific bioinks, key challenges remain.[139] These include improving long-term stability, vascular permeability, and standardization for reproducibility.[23,74] Future efforts should focus on developing modular, tunable

Table 1. Summary of representative bioinks used in skeletal muscle bioprinting

Bioink type	Advantages	Limitations	References
Natural (GelMA, collagen, fibrin, dECM)	High bioactivity; promotes myogenesis; tissue-specific signals	Poor mechanical strength; variability; fast degradation	[31,79]
Synthetic (PCL, PEGDA, PLA)	Strong mechanical properties; slow degradation	Low bioactivity; needs modification; may reduce viability	[91,92]
Composite (GelMA + PCL, PEGDA + Collagen)	Combines bioactivity and mechanical strength; tunable	Complex preparation; potential crosslinking issues	[99,103]
Biofunctional (with IGF-1, VEGF, NGF)	Enhances differentiation, angiogenesis, and innervation	Dose control; short half-life; potential toxicity	[115,138]
Nanomaterial-enhanced (CNTs, GO, AuNPs)	Improves conductivity, alignment, mechanical reinforcement	Stability; dispersibility; possible immune reactions	[121,124]
4D/stimuli-responsive	Dynamic response to stimuli; mimics physiological behavior	Immature technology; complex fabrication	[130,131]

Abbreviations: AuNPs, gold nanoparticles; CNTs, carbon nanotubes; dECM, decellularized extracellular matrix; GelMA, gelatin methacrylate; GO, graphene oxide; IGF-1, insulin-like growth factor-1; NGF, nerve growth factor; PCL, polycaprolactone; PEGDA, polyethylene glycol diacrylate; PLA, polylactic acid; VEGF, vascular endothelial growth factor.

bioink libraries that can be tailored to patient-specific needs and integrated with real-time monitoring systems for adaptive printing.

Combining biological complexity, mechanical integrity, and functional responsiveness in a single bioink platform will be critical to advancing the clinical translation of skeletal muscle bioprinting.[20,26]

5. Bioprinting strategies, functional validation, and future directions

The fabrication of functional skeletal muscle tissue requires bioprinting approaches capable of replicating its complex anisotropic architecture, multicellular composition, and dynamic responsiveness.[20,110,140] Different bioprinting modalities offer varying degrees of spatial control, resolution, and material compatibility. This section systematically reviews both conventional and emerging bioprinting technologies, with a focus on their applicability to muscle tissue engineering.[141,142] Figure 3 illustrates a representative workflow of skeletal muscle bioprinting, encompassing cell selection, bioink preparation, construct fabrication, and post-printing maturation.

5.1. Bioprinting platforms and construct design

Bioprinting of skeletal muscle constructs leverages several key platforms, each offering unique advantages and limitations. Among them, extrusion-based bioprinting is the most widely adopted, enabling the deposition of high-viscosity, cell-laden bioinks that support fiber alignment—essential for restoring contractility—despite challenges related to resolution and cell shear stress.[71,140,143–150] Inkjet bioprinting provides high precision and rapid droplet-based patterning, making it suitable for growth factor distribution or single-cell placement; however, it is limited by ink viscosity and potential thermal or mechanical stress on cells.[151–159] Laser-assisted bioprinting, particularly laser-induced forward transfer, offers excellent resolution and cell viability for microscale patterning of neural or vascular cells, though it remains costly and technically demanding.[160–163] Emerging platforms such as coaxial extrusion and microfluidic-assisted bioprinting have expanded the design space by enabling spatially organized, perfusable structures and real-time control of bioink composition, providing new opportunities for muscle tissue engineering.[110,164–169] Digital light processing bioprinting employs photopolymerization via patterned

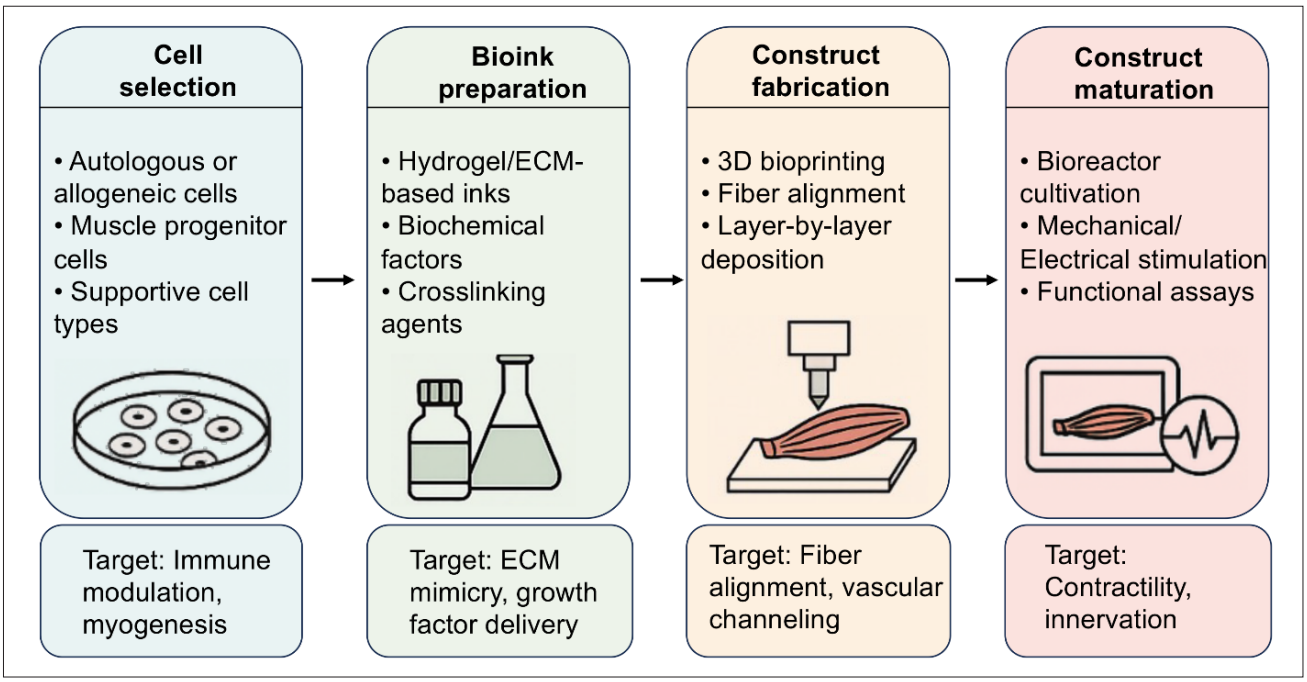

Figure 3. Workflow of skeletal muscle bioprinting, from cell selection to construct maturation. The process includes four key stages: (i) Cell selection—autologous or allogeneic cells, including muscle progenitor and supportive cell types; (ii) Bioink preparation—hydrogel/ extracellular matrix (ECM)-based inks supplemented with biochemical cues and crosslinking agents to mimic the native muscle microenvironment; (iii) Construct fabrication—3D bioprinting (e.g., extrusion-based) to enable fiber alignment and layer-by-layer deposition; (iv) Construct maturation—bioreactor culture, mechanical/ electrical stimulation, and functional assays to promote tissue development and contractility. Each stage is annotated with corresponding biological targets—such as immune modulation, myogenesis, ECM mimicry, fiber alignment, and functional restoration—to emphasize the alignment between engineering strategies and regenerative outcomes.

light exposure, supporting rapid fabrication of high-resolution, multilayered constructs when paired with suitable photo-crosslinkable bioinks.[170,171] These advanced modalities collectively enhance spatial resolution, cell viability, and structural complexity, enabling the creation of muscle tissues with improved biomimetic features.[172] To better contextualize the strengths and limitations of each bioprinting platform, Table 2 provides a comparative overview of key parameters relevant to skeletal muscle tissue engineering applications.

Achieving physiological function in engineered skeletal muscle constructs requires not only structural accuracy but also cellular alignment and maturation.[138,177] Multiple strategies have been implemented to guide fiber orientation and promote myogenic differentiation post-printing.[76,178] The application of electrical or magnetic fields has been shown to direct cytoskeletal alignment and enhance the expression of myogenic genes.[179,180] Topographical patterning through microgrooved substrates or surface templating can guide cell alignment by providing physical cues that mimic the native ECM architecture.[181] Additionally, dynamic culture in bioreactors—applying cyclic mechanical stretching or electrical stimulation—significantly improves sarcomere organization, contractile function, and metabolic activity in engineered muscle tissues.[182] These adjunctive techniques are often integrated during the post-printing phase and are critical for transforming printed constructs into physiologically responsive muscle analogs.

To facilitate clinical translation, muscle constructs must be scalable, vascularizable, and compatible with host integration.[183] Key design considerations include the incorporation of perfusable microchannels or sacrificial templates to overcome diffusion limitations in thick tissues, as well as the spatial organization of myogenic, endothelial, and neural cells to replicate the hierarchical structure of native muscle.[19,56]

Moreover, the selected printing modality must align with downstream processing requirements such as in situ bioprinting, bioreactor conditioning, or integration with wearable bioelectronics.[184–186] Regulatory considerations, including batch consistency, sterilizability, and manufacturing scalability, must also be addressed early in the design process.[187,188] Through the integration of advanced bioprinting platforms and biologically informed design strategies, skeletal muscle constructs can increasingly approach clinical-grade complexity and functionality.

A critical step in validating engineered skeletal muscle constructs is the rigorous assessment of their biological functionality.[189] Both *in vitro* and *in vivo* evaluations are essential to determine the myogenic potential, integration capability, and regenerative efficacy of bioprinted tissues.[19,59,138] The following sections review current

Table 2. Comparative analysis of bioprinting technologies for skeletal muscle engineering

Bioprinting Technology	Spatial resolution (μm)	Post-print cell viability (%)	Bioink compatibility	Advantages	Limitations	References
Extrusion-based	~ 100–400 (typical); best 105 ± 9	No significant effect	High-viscosity, cell-laden inks	Widely used; supports cell-laden viscous inks; good for fiber alignment	Low resolution; potential cell shear stress	140,158,143,145,148
Inkjet-based	20–50	85–98	Low-viscosity, low-cell-density	High precision; low cost; ideal for growth factor or cell patterning	Not suitable for viscous or dense inks	153,156,157
Laser-assisted	<10	90–100	Moderate viscosity, specialized formulation	High resolution and cell viability; precise droplet placement	High cost and complexity; limited ink options	160,161,173
Coaxial extrusion	~100	≥80	Multi-material, core–shell inks	Enables aligned fiber and vessel-like channels; supports multi-phase structures	Complex nozzle setup; scalability challenges	174,165,164
Microfluidic-assisted	50–100	85–95	Gradient or multi-cell bioinks	Fine control of mixing and gradients; spatial patterning	Requires precise flow control; complex setup	167–169,175
Digital light processing	10–50	80–93	Photocrosslinkable bioinks	High speed and resolution; enables complex shape fabrication	Limited bioink options; potential phototoxicity	176

methodologies for functional testing and summarize key outcomes from preclinical models.

5.2. *In vitro* evaluation of engineered muscle

In vitro testing provides initial insights into the viability, differentiation, and contractile behavior of printed muscle constructs.[20,177,190,191] To evaluate post-printing cytocompatibility, cell viability is commonly assessed via live/dead staining, metabolic activity assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or Alamar Blue, and proliferation marker analysis.[192–194]

Myogenic differentiation is evaluated through the expression of lineage-specific markers such as myoblast determination protein 1, myogenin, and myosin heavy chain, using techniques like quantitative polymerase chain reaction, immunofluorescence, and western blotting.[195,196] Alignment and fusion of myoblasts into multinucleated myotubes are considered indicative of successful myogenic maturation.[177]

Functional assessment includes calcium imaging to detect intracellular calcium transients associated with contraction, as well as force generation analysis via electrical stimulation.[177,197,198] These assays provide important indicators of sarcomere formation, excitation–contraction coupling, and overall muscle construct performance at the cellular level.

In addition to assessing regenerative potential, 3D-bioprinted skeletal muscle is now deployed as a human, disease-relevant test-bed. When muscle progenitors isolated from Duchenne muscular dystrophy patients are printed into aligned, contractile bundles, the tissues faithfully reproduce sarcolemmal fragility, reduced twitch force, impaired myotube maturation, and profibrotic extracellular-matrix deposition—hallmarks of the *in vivo* pathology.[199,200] These patient-specific constructs furnish a scalable alternative to animal models for high-throughput screening. "Myobundles" engineered from healthy adult cells hypertrophy in response to IGF-1, yet atrophy after glucocorticoid exposure, mirroring clinical drug responses, while the same platform has recently revealed taxane-induced myotoxicity mechanisms.[201,202] Integrating stretchable piezoresistive hydrogel networks or optogenetic actuators enables non-invasive, real-time monitoring of force generation, calcium transients, and fatigue during pharmacological challenges, further expanding analytical throughput.[203,204] In parallel, Loi *et al.*[205] have designed a bioprinting-integrated cyclic-stretch bioreactor that markedly enhances myogenic maturation of printed constructs. By uniting structural fidelity, patient specificity, and dynamic biosensing, bioprinted skeletal muscle platforms are rapidly maturing into versatile tools for mechanistic discovery and translational drug development.

5.3. *In vivo* validation and translational considerations

Preclinical validation of bioprinted skeletal muscle constructs predominantly relies on VML models, most commonly established in rodents.[198,206] These models involve the surgical excision of a critical volume of muscle tissue that cannot regenerate spontaneously, thereby simulating clinical scenarios such as trauma or tumor resection.[207–209]

Implanted constructs are evaluated for their capacity to integrate with host tissue, restore morphology, and re-establish vascular and neural connections.[59,210] Histological staining, such as hematoxylin and eosin, Masson's trichrome, and immunolabeling of endothelial and neural markers, is used to assess structural integration, fibrosis, and cell fate.[183,211] Functional recovery is measured through a combination of force output testing, typically via *in situ* or *ex vivo* muscle contractility assays, and behavioral metrics such as gait analysis, limb drag, or treadmill performance.[212–215] Electrophysiological testing is also employed to assess neuromuscular junction formation and stimulus-responsive contraction.[206,216] While these models provide crucial data, inter-study variability in defect size, implantation methods, evaluation timelines, and functional benchmarks remains a challenge for reproducibility and translational extrapolation.[14,115,217,218]

Recent studies have reported encouraging results in which bioprinted constructs incorporating aligned myotubes, vascular endothelial cells, and neurogenic components have achieved partial restoration of muscle structure and function. Constructs with hierarchical alignment and prevascularization show improved integration and a reduced fibrotic response.[138,210,219,220] Despite these advancements, complete functional restoration equivalent to native muscle remains elusive. Challenges include insufficient long-term innervation, limited mechanical strength, immune responses in immunocompetent models, and a lack of contractile endurance over time.[12,221–223] Additionally, the absence of standardized protocols for assessing recovery makes cross-study comparisons difficult.[224]

To address these limitations, emerging research is focusing on dynamic *in vitro* maturation platforms, humanized animal models, and long-term follow-up protocols that better emulate clinical scenarios.[225] Furthermore, integration with biosensors and real-time monitoring tools is expected to enhance functional readouts and accelerate clinical readiness.[226] Recent advances in large-animal studies further enhance

translational relevance. For example, bioprinted skeletal muscle constructs larger than 3 cm^3, implanted in rabbits and mini-pigs, have restored 55–70% of tetanic force and demonstrated macroscopically evident vascular and neuromuscular integration.[227,228] In parallel, perfused human muscle slices and patient-derived myobundles have maintained contractile and electrophysiological function for more than 28 days *in vitro*, providing medium-throughput platforms for drug screening and personalized therapy evaluation.[229] Collectively, these two tiers of validation—large-animal functional recovery and human-relevant *in vitro* analytics—align with ISO 10993-6 and ASTM F3224 guidelines, thereby reducing translational risk and meeting key regulatory expectations for first-in-human applications.

6. Future directions and translational outlook

6.1. Multi-tissue integration for physiological fidelity

Bioprinting has significantly advanced our ability to engineer biomimetic skeletal muscle tissues; however, several formidable challenges continue to constrain clinical translation. Current constructs, while demonstrating partial functional restoration in preclinical models, often fail to achieve comprehensive neurovascular integration, sustained mechanical and contractile performance, and long-term tissue remodeling under physiological conditions.[28,230,231] In addition, variability in cell sources, bioink compositions, and assessment protocols hinders cross-study comparability and reproducibility.

From a technical perspective, scale-up manufacturing remains a critical bottleneck.[232] Translating microscale constructs into clinically relevant dimensions demands novel strategies to ensure structural fidelity, cell viability, and mass transport in thicker tissues.[19,20] Moreover, standardized functional benchmarks—including force generation, fatigue resistance, and neuromuscular responsiveness—are urgently needed to facilitate regulatory approval and interlaboratory validation.[205]

6.2. High-throughput functional analytics and artificial intelligence

Emerging technologies are reshaping the landscape of muscle bioprinting. Four-dimensional (4D; time-responsive) bioprinting, which enables time-responsive structural transformations, holds promise for generating constructs capable of dynamic adaptation to biomechanical and biochemical cues *in vivo*—mimicking developmental myogenesis or injury-induced remodeling.[205] Simultaneously, artificial intelligence (AI) and machine learning are revolutionizing design and optimization, offering predictive tools for bioink formulation, anisotropic print path design, and functional outcome modeling.[233,234] Coupled with high-throughput pipelines, AI-guided systems can reduce empirical iterations and accelerate progress toward clinically viable constructs.[235,236] Figure 4 illustrates an integrated, future-oriented muscle bioprinting system that incorporates these emerging components into a unified pipeline.

Integration with bioelectronic and rehabilitation interfaces marks another promising direction. Implantable sensors, neuromuscular stimulators, and stretchable electrodes can establish closed-loop feedback systems to enhance post-implantation adaptation, monitor inflammation, and track host–graft interactions in real time.[237,238] Looking ahead, next-generation muscle bioprinting platforms will likely converge on modular, patient-specific solutions integrating smart biomaterials, autologous or gene-edited cells, and tunable microenvironments.[239] These platforms must not only replicate muscle structure but also support immune compatibility, long-term remodeling, and adaptive rehabilitation.[20] To achieve clinical translation, the field must shift toward standardized, scalable, and regulatory-compliant protocols, enabled by close collaboration across materials science, bioengineering, and clinical disciplines.[188]

6.3. Clinical translation: trials, regulation, and scalability

Despite preclinical success, no skeletal muscle bioprinting trial has yet been initiated in a clinical setting; a recent scoping review found only 11 registered bioprinting studies worldwide, all targeting other tissues.[240] In most jurisdictions, printed, cell-laden muscle grafts will be regulated as combination products (FDA: Technical Considerations for Additive Manufactured Medical Devices, 2021) or as ATMPs (EMA: Regulation (EC) No. 1394/2007 on ATMPs), demanding rigorous demonstration of safety, potency, and manufacturing consistency. Key scalability bottlenecks—including kilogram-scale cell sourcing, perfusable vascular/neuronal networks, closed-loop GMP bioprinting, and cost-effective bioink standardization—remain unresolved.[188] Addressing these intertwined clinical, regulatory, and manufacturing challenges will be critical for moving bioprinted skeletal-muscle constructs from bench to bedside.

7. Conclusion

Bioprinting has rapidly evolved into a transformative strategy for skeletal muscle regeneration, enabling the precise construction of spatially organized, biologically functional, and structurally aligned muscle tissues. Recent

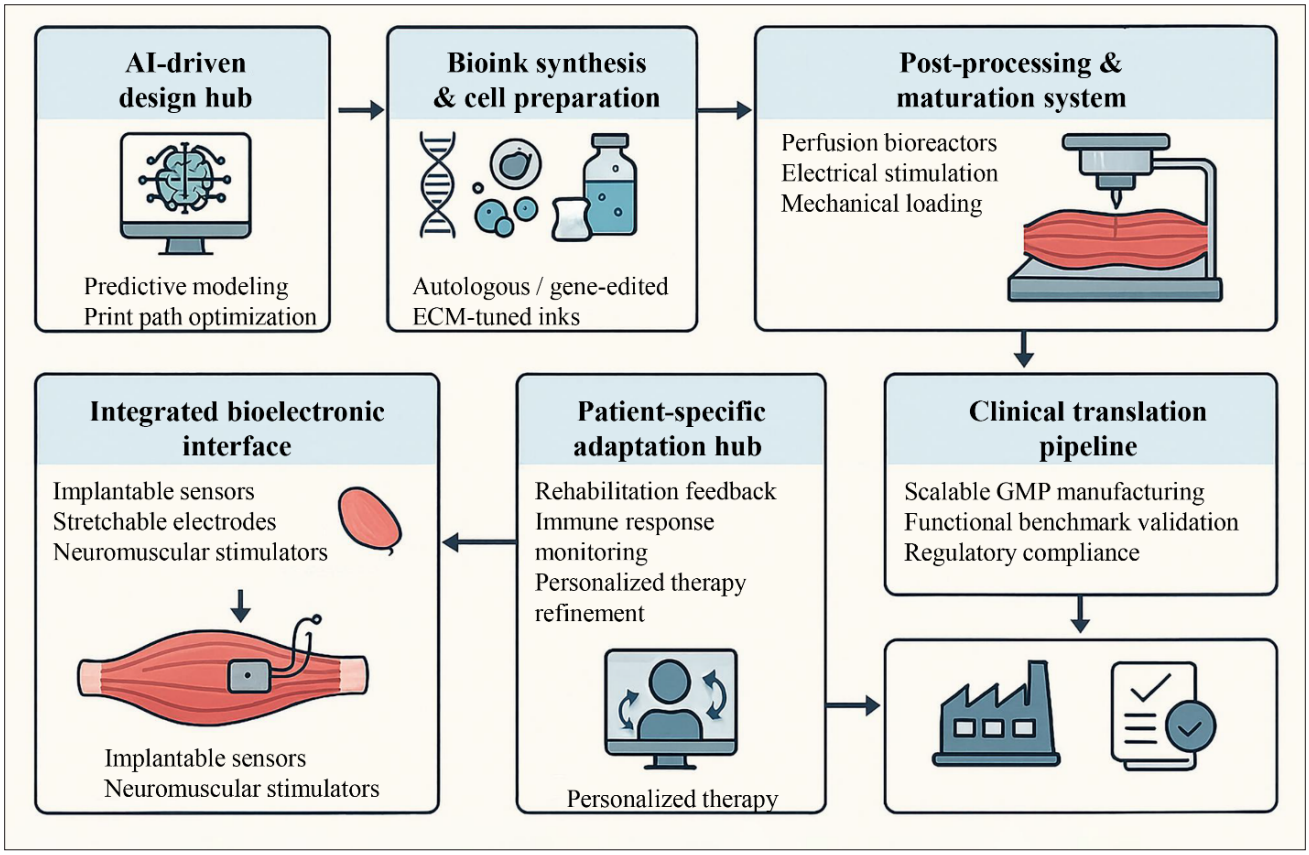

Figure 4. Conceptual illustration of an integrated future-oriented muscle bioprinting system. The diagram outlines a modular workflow integrating AI-guided design, smart bioink and cell preparation, 3D/4D bioprinting, and post-printing maturation. Key components include bioelectronic interfaces for real-time monitoring, patient-specific adaptation hubs, and a clinical translation pipeline supporting scalable, regulatory-compliant manufacturing. This system highlights the convergence of emerging technologies toward personalized, functional skeletal muscle regeneration. Abbreviations: AI, artificial intelligence; ECM, extracellular matrix; GMP, good manufacturing practice.

advances in biomaterials science, cellular engineering, and printing technologies have significantly enhanced the fidelity and functionality of engineered muscle constructs. However, several substantial challenges remain, particularly the achievement of full neurovascular integration, functional restoration comparable to native tissues, and the development of scalable manufacturing processes for clinical application. These challenges highlight the need for standardized evaluation protocols, robust bioink formulations, and interdisciplinary design principles that integrate biology, mechanics, and architecture. Future research must focus on developing dynamic, bioresponsive systems capable not only of replicating structural anisotropy but also of interacting with host physiology in a feedback-responsive manner. The integration of 4D bioprinting, machine learning-based optimization, and electromechanical stimulation technologies will be critical for advancing personalized and adaptive muscle repair strategies. By aligning material innovation with clinical needs, skeletal muscle bioprinting is poised for clinical translation. Continued cross-disciplinary collaboration will be essential in transforming current proof-of-concept studies into standardized, regulatory-compliant, and patient-specific therapies, ultimately revolutionizing the field of musculoskeletal regenerative medicine.

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Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

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References

1. Relaix F, Bencze M, Borok MJ, *et al*. Perspectives on skeletal muscle stem cells. *Nat Commun*. 2021;12(1):692. doi: 10.1038/s41467-020-20760-6
2. Judson RN, Rossi FMV. Towards stem cell therapies for skeletal muscle repair. *NJP Regen Med*. 2020;5(1):10. doi: 10.1038/s41536-020-0094-3
3. Sousa-Victor P, García-Prat L, Muñoz-Cánoves P. Control of satellite cell function in muscle regeneration and its disruption in ageing. *Nat Rev Mol Cell Bio*. 2022;23(3):204-226. doi: 10.1038/s41580-021-00421-2
4. Qi L, Zhang F, Wang K, *et al*. Advancements in skeletal muscle tissue engineering: Strategies for repair and regeneration of skeletal muscle beyond self-repair. *Regen Biomater*. 2025;12:rbaf050. doi: 10.1093/rb/rbaf050
5. Maimaiti D, Ge X, Wang C, *et al*. Extracellular matrix-mimicking cryogels composed of methacrylated fucoidan enhance vascularized skeletal muscle regeneration following volumetric muscle loss. *Int J Biol Macromol*. 2024;283:137122. doi: 10.1016/j.ijbiomac.2024.137122
6. Sonaye SY, Sikder P. Bioengineered constructs as a tissue engineering-based therapy for volumetric muscle loss. *Tissue Eng Part B Rev*. 2025. doi: 10.1089/ten.teb.2025.0017
7. Cai CW, Grey JA, Hubmacher D, Han WM. Biomaterial-based regenerative strategies for volumetric muscle loss: challenges and solutions. *Adv Wound Care*. 2025;14(3):159-175. doi: 10.1089/wound.2024.0079
8. Edouard P, Reurink G, Mackey AL, *et al*. Traumatic muscle injury. *Nat Rev Dis Primers*. 2023;9(1):56. doi: 10.1038/s41572-023-00469-8
9. SantAnna JPC, Pedrinelli A, Hernandez AJ, Fernandes TL. Muscle injury: pathophysiology, diagnosis, and treatment. *Rev Bras Ortop*. 2022;57(1):1-13. doi: 10.1055/s-0041-1731417
10. Topaloğlu H, Poorshiri B. The congenital muscular dystrophies. *Ann Child Neurol Soc*. 2024;2(1):27-39. doi: 10.1002/cns3.20050
11. Ostojic M, Indelli PF, Lovrekovic B, *et al*. Graft selection in anterior cruciate ligament reconstruction: a comprehensive review of current trends. *Medicina*. 2024;60(12):2090. doi: 10.3390/medicina60122090
12. Gahlawat S, Oruc D, Paul N, *et al*. Tissue engineered 3D constructs for volumetric muscle loss. *Ann Biomed Eng*. 2024;52(9):2325-2347. doi: 10.1007/s10439-024-03541-w
13. Boyer O, Butler-Browne G, Chinoy H, *et al*. Myogenic cell transplantation in genetic and acquired diseases of skeletal muscle. *Front Genet*. 2021;12. doi: 10.3389/fgene.2021.702547
14. Liu H, Cheema U, Player DJ. Photobiomodulation therapy (PBMT) in skeletal muscle regeneration: a comprehensive review of mechanisms, clinical applications, and future directions. *Photodiagn Photodyn*. 2025;53:104634. doi: 10.1016/j.pdpdt.2025.104634
15. de Sire A, Marotta N, Lippi L, *et al*. Pharmacological treatment for acute traumatic musculoskeletal pain in athletes. *Medicina*. 2021;57(11):1208. doi: 10.3390/medicina57111208
16. Wei Q, An Y, Zhao X, Li M, Zhang J. Three-dimensional bioprinting of tissue-engineered skin: biomaterials, fabrication techniques, challenging difficulties, and future directions: a review. *Int J Biol Macromol*. 2024;266:131281. doi: 10.1016/j.ijbiomac.2024.131281
17. Saini G, Segaran N, Mayer JL, Saini A, Albadawi H, Oklu R. Applications of 3D bioprinting in tissue engineering and regenerative medicine. *J Clin Med*. 2021;10(21):4966. doi: 10.3390/jcm10214966

18. Jain P, Kathuria H, Dubey N. Advances in 3D bioprinting of tissues/organs for regenerative medicine and *in-vitro* models. *Biomaterials*. 2022;287:121639. doi: 10.1016/j.biomaterials.2022.121639

19. Samandari M, Quint J, Rodríguez-delaRosa A, Sinha I, Pourquié O, Tamayol A. Bioinks and bioprinting strategies for skeletal muscle tissue engineering. *Adv Mater*. 2022;34(12):2105883. doi: 10.1002/adma.202105883

20. Sabetkish S, Currie P, Meagher L. Recent trends in 3D bioprinting technology for skeletal muscle regeneration. *Acta Biomater*. 2024;181:46-66. doi: 10.1016/j.actbio.2024.04.038

21. Lee H, Kim W, Lee J, *et al*. Self-aligned myofibers in 3D bioprinted extracellular matrix-based construct accelerate skeletal muscle function restoration. *Appl Phys Rev*. 2021;8(2):021405. doi: 10.1063/5.0039639

22. Sicherer ST, Haque N, Parikh Y, Grasman JM. Current methodologies for inducing aligned myofibers in tissue constructs for skeletal muscle tissue regeneration. *Adv Wound Care*. 2025;14(2):114-131. doi: 10.1089/wound.2024.0111

23. Zhang H, Wu C. 3D printing of biomaterials for vascularized and innervated tissue regeneration. *Int J Bioprinting*. 2023;9(3):706. doi: 10.18063/ijb.706

24. Thangadurai M, Ajith A, Budharaju H, Sethuraman S, Sundaramurthi D. Advances in electrospinning and 3D bioprinting strategies to enhance functional regeneration of skeletal muscle tissue. *Biomater Adv*. 2022;142:213135. doi: 10.1016/j.bioadv.2022.213135

25. Miramini S, Fegan KL, Green NC, Espino DM, Zhang L, Thomas-Seale LEJ. The status and challenges of replicating the mechanical properties of connective tissues using additive manufacturing. *J Mech Behav Biomed*. 2020;103:103544. doi: 10.1016/j.jmbbm.2019.103544

26. García-Lizarribar A, Villasante A, Lopez-Martin JA, *et al*. 3D bioprinted functional skeletal muscle models have potential applications for studies of muscle wasting in cancer cachexia. *Biomater Adv*. 2023;150:213426. doi: 10.1016/j.bioadv.2023.213426

27. Alave Reyes-Furrer A, De Andrade S, Bachmann D, *et al*. Matrigel 3D bioprinting of contractile human skeletal muscle models recapitulating exercise and pharmacological responses. *Commun Biol*. 2021;4(1):1183. doi: 10.1038/s42003-021-02691-0

28. Mancuso S, Bhalerao A, Cucullo L. Advances and challenges of bioassembly strategies in neurovascular in vitro modeling: an overview of current technologies with a focus on three-dimensional bioprinting. *Int J Mol Sci*. 2024;25(20):11000. doi: 10.3390/ijms252011000

29. Lee SJ, Jeong W, Atala A. 3D bioprinting for engineered tissue constructs and patient-specific models: current progress and prospects in clinical applications. *Adv Mater*. 2024;36(49):e2408032. doi: 10.1002/adma.202408032

30. Mathur V, Agarwal P, Kasturi M, Srinivasan V, Seetharam RN, Vasanthan KS. Innovative bioinks for 3D bioprinting: exploring technological potential and regulatory challenges. *J Tissue Eng*. 2025;16:20417314241308022. doi: 10.1177/20417314241308022

31. Liu H, Xing F, Yu P, *et al*. Biomimetic fabrication bioprinting strategies based on decellularized extracellular matrix for musculoskeletal tissue regeneration: current status and future perspectives. *Mater Des*. 2024;243:113072. doi: 10.1016/j.matdes.2024.113072

32. Chu J, Lu M, Pfeifer CG, Alt V, Docheva D. Rebuilding tendons: a concise review on the potential of dermal fibroblasts. *Cells-basel*. 2020;9(9):2047. doi: 10.3390/cells9092047

33. Stocco TD, Zhang T, Dimitrov E, *et al*. Carbon nanomaterial-based hydrogels as scaffolds in tissue engineering: a comprehensive review. *Int J Nanomed*. 2023;18:6153-6183. doi: 10.2147/IJN.S436867

34. Brooks SV, Guzman SD, Ruiz LP. Skeletal muscle structure, physiology, and function. *Handb Clin Neurol*. 2023;195:3-16. doi: 10.1016/b978-0-323-98818-6.00013-3

35. Feng LT, Chen ZN, Bian H. Skeletal muscle: molecular structure, myogenesis, biological functions, and diseases. *MedComm*. 2024;5(7):e649. doi: 10.1002/mco2.649

36. Roy BC, Bruce HL. Contribution of intramuscular connective tissue and its structural components on meat tenderness-revisited: a review. *Crit Rev Food Sci*. 2024;64(25):9280-9310. doi: 10.1080/10408398.2023.2211671

37. Sunadome K, Erickson AG, Kah D, *et al*. Directionality of developing skeletal muscles is set by mechanical forces. *Nat Commun*. 2023;14(1):3060. doi: 10.1038/s41467-023-38647-7

38. Wohlgemuth RP, Brashear SE, Smith LR. Alignment, cross linking, and beyond: a collagen architect's guide to the skeletal muscle extracellular matrix. *Am J Physiol Cell Physiol*. 2023;325(4):C1017-C1030. doi: 10.1152/ajpcell.00287.2023

39. Kim W, Kim G. Bioprinting 3D muscle tissue supplemented with endothelial-spheroids for neuromuscular junction model. *Appl Phys Rev*. 2023;10(3):031410. doi: 10.1063/5.0152924

40. Rudolf R, Kettelhut IC, Navegantes LCC. Sympathetic innervation in skeletal muscle and its role at the neuromuscular junction. *J Muscle Res Cell Motil*. 2024;45(2):79-86. doi: 10.1007/s10974-024-09665-9

41. Poole DC, Kano Y, Koga S, Musch TI. August krogh: muscle capillary function and oxygen delivery. *Comp Biochem Physiol A Mol Integr Physiol*. 2021;253:110852. doi: 10.1016/j.cbpa.2020.110852

42. Mondrinos MJ, Alisafaei F, Yi AY, *et al*. Surface-directed engineering of tissue anisotropy in microphysiological models of musculoskeletal tissue. *Sci Adv*. 2021;7(11):eabe9446. doi: 10.1126/sciadv.abe9446

43. Ferrara PJ, Yee EM, Petrocelli JJ, *et al*. Macrophage immunomodulation accelerates skeletal muscle functional recovery in aged mice following disuse atrophy. *J Appl Physiol*. 2022;133(4):919-931. doi: 10.1152/japplphysiol.00374.2022

44. Wirth G, Juusola G, Tarvainen S, Laakkonen JP, Korpisalo P, Ylä-Herttuala S. Capillary dynamics regulate post-ischemic muscle damage and regeneration in experimental hindlimb ischemia. *Cells Basel*. 2023;12(16):2060. doi: 10.3390/cells12162060

45. Pajalunga D, Crescenzi M. Restoring the cell cycle and proliferation competence in terminally differentiated skeletal muscle myotubes. *Cells Basel*. 2021;10(10):2753. doi: 10.3390/cells10102753

46. Fukada S ichiro, Higashimoto T, Kaneshige A. Differences in muscle satellite cell dynamics during muscle hypertrophy and regeneration. *Skelet Muscle*. 2022;12(1):17. doi: 10.1186/s13395-022-00300-0

47. Andre AB, Rees KP, O'Connor S, *et al*. Single cell analysis reveals satellite cell heterogeneity for proinflammatory chemokine expression. *Front Cell Dev Biol*. 2023;11. doi: 10.3389/fcell.2023.1084068

48. Zhong T, Gao N, Niu H, *et al*. Targeted delivery of engineered extracellular vesicles to simultaneously promote vascularization and muscle regeneration in ischemic limbs. *J Control Release*. 2025;384:113938. doi: 10.1016/j.jconrel.2025.113938

49. Wang X, Zhou L. The many roles of macrophages in skeletal muscle injury and repair. *Front Cell Dev Biol*. 2022;10. doi: 10.3389/fcell.2022.952249

50. Wang X, Zhou L. The multifaceted role of macrophages in homeostatic and injured skeletal muscle. *Front Immunol*. 2023;14. doi: 10.3389/fimmu.2023.1274816

51. Zelada D, Bermedo-García F, Collao N, Henríquez JP. Motor function recovery: Deciphering a regenerative niche at the neuromuscular synapse. *Biol Rev*. 2021;96(2):752-766. doi: 10.1111/brv.12675

52. Guzman SD, Brooks SV. Skeletal muscle innervation: reactive oxygen species as regulators of neuromuscular junction dynamics and motor unit remodeling. *Free Radical Bio Med*. 2025;230:58-65. doi: 10.1016/j.freeradbiomed.2025.01.053

53. Cordelle MZ, Snelling SJB, Mouthuy PA. Skeletal muscle tissue engineering: from tissue regeneration to biorobotics. *Cyborg Bionic Syst*. 2025;6:0279. doi: 10.34133/cbsystems.0279

54. Langridge B, Griffin M, Butler PE. Regenerative medicine for skeletal muscle loss: a review of current tissue engineering approaches. *J Mater Sci-mater M*. 2021;32(1):15. doi: 10.1007/s10856-020-06476-5

55. Xing J, Liu N, Xu N, Chen W, Xing D. Engineering complex anisotropic scaffolds beyond simply uniaxial alignment for tissue engineering. *Adv Funct Mater*. 2022;32(15):2110676. doi: 10.1002/adfm.202110676

56. Filippi M, Yasa O, Giachino J, *et al*. Perfusable biohybrid designs for bioprinted skeletal muscle tissue. *Adv Healthc Mater*. 2023;12(18):2300151. doi: 10.1002/adhm.202300151

57. Shiwarski DJ, Hudson AR, Tashman JW, *et al*. 3D bioprinting of collagen-based high-resolution internally perfusable scaffolds for engineering fully biologic tissue systems. *Sci Adv*. 2025;11(17):eadu5905. doi: 10.1126/sciadv.adu5905

58. Gordon T. Peripheral nerve regeneration and muscle reinnervation. *Int J Mol Sci*. 2020;21(22):8652. doi: 10.3390/ijms21228652

59. Kim JH, Kim I, Seol YJ, *et al*. Neural cell integration into 3D bioprinted skeletal muscle constructs accelerates restoration of muscle function. *Nat Commun*. 2020;11(1):1025. doi: 10.1038/s41467-020-14930-9

60. Christensen KW, Turner J, Coughenour K, *et al*. Assembled cell-decorated collagen (AC-DC) fiber bioprinted implants with musculoskeletal tissue properties promote functional recovery in volumetric muscle loss. *Adv Healthc Mater*. 2022;11(3):2101357. doi: 10.1002/adhm.202101357

61. Park W, Gao G, Cho DW. Tissue-specific decellularized extracellular matrix bioinks for musculoskeletal tissue regeneration and modeling using 3D bioprinting technology. *Int J Mol Sci*. 2021;22(15):7837. doi: 10.3390/ijms22157837

62. Cakal SD, Radeke C, Alcala JF, *et al*. A simple and scalable 3D printing methodology for generating aligned and extended human and murine skeletal muscle tissues. *Biomed Mater*. 2022;17(4):045013. doi: 10.1088/1748-605X/ac6b71

63. Volpi M, Paradiso A, Costantini M, Święszkowski W. Hydrogel-based fiber biofabrication techniques for skeletal muscle tissue engineering. *ACS Biomater Sci Eng*. 2022;8(2):379-405. doi: 10.1021/acsbiomaterials.1c01145

64. Baiguera S, Del Gaudio C, Di Nardo P, Manzari V, Carotenuto F, Teodori L. 3D printing decellularized extracellular matrix

to design biomimetic scaffolds for skeletal muscle tissue engineering. *BioMed Res Int*. 2020;2020(1):2689701. doi: 10.1155/2020/2689701

65. Bertassoni LE. Bioprinting of complex multicellular organs with advanced functionality—recent progress and challenges ahead. *Adv Mater*. 2022;34(3):2101321. doi: 10.1002/adma.202101321

66. Zhuang P, An J, Chua CK, Tan LP. Bioprinting of 3D in vitro skeletal muscle models: a review. *Mater Des*. 2020;193:108794. doi: 10.1016/j.matdes.2020.108794

67. Ronzoni FL, Aliberti F, Scocozza F, *et al*. Myoblast 3D bioprinting to burst in vitro skeletal muscle differentiation. *J Tissue Eng Regen Med*. 2022;16(5):484-495. doi: 10.1002/term.3293

68. Kim WJ, Kim GH. 3D bioprinting of functional cell-laden bioinks and its application for cell-alignment and maturation. *Appl Mater Today*. 2020;19:100588. doi: 10.1016/j.apmt.2020.100588

69. Gilbert-Honick J, Grayson W. Vascularized and innervated skeletal muscle tissue engineering. *Adv Healthc Mater*. 2020;9(1):1900626. doi: 10.1002/adhm.201900626

70. Blake C, Massey O, Boyd-Moss M, *et al*. Replace and repair: biomimetic bioprinting for effective muscle engineering. *APL Bioeng*. 2021;5(3):031502. doi: 10.1063/5.0040764

71. Davoodi E, Sarikhani E, Montazerian H, *et al*. Extrusion and microfluidic-based bioprinting to fabricate biomimetic tissues and organs. *Adv Mater Technol*. 2020;5(8):1901044. doi: 10.1002/admt.201901044

72. Abrishamkar A, Nilghaz A, Saadatmand M, Naeimirad M, deMello AJ. Microfluidic-assisted fiber production: potentials, limitations, and prospects. *Biomicrofluidics*. 2022;16(6):061504. doi: 10.1063/5.0129108

73. Adhikari J, Roy A, Das A, *et al*. Effects of processing parameters of 3D bioprinting on the cellular activity of bioinks. *Macromol Biosci*. 2021;21(1):2000179. doi: 10.1002/mabi.202000179

74. Ngan CGY, Quigley A, Williams RJ, *et al*. Matured myofibers in bioprinted constructs with in vivo vascularization and innervation. *Gels Basel*. 2021;7(4):171. doi: 10.3390/gels7040171

75. Kang MS, Lee SH, Park WJ, Lee JE, Kim B, Han DW. Advanced techniques for skeletal muscle tissue engineering and regeneration. *Bioengineering*. 2020;7(3):99. doi: 10.3390/bioengineering7030099

76. Lee S, Kim W, Kim G. Efficient myogenic activities achieved through blade-casting-assisted bioprinting of aligned myoblasts laden in collagen bioink. *Biomacromolecules*. 2023;24(11):5219-5229. doi: 10.1021/acs.biomac.3c00749

77. Xiaorui L, Fuyin Z, Xudong W, *et al*. Biomaterial inks for extrusion-based 3D bioprinting: Property, classification, modification, and selection. *Int J Bioprinting*. 2022;9(2):649. doi: 10.18063/ijb.v9i2.649

78. Smoak MM, Hogan KJ, Grande-Allen KJ, Mikos AG. Bioinspired electrospun dECM scaffolds guide cell growth and control the formation of myotubes. *Sci Adv*. 2021;7(20):eabg4123. doi: 10.1126/sciadv.abg4123

79. Hwangbo H, Lee H, Jin EJ, *et al*. Bio-printing of aligned GelMa-based cell-laden structure for muscle tissue regeneration. *Bioact Mater*. 2022;8(1):57-70. doi: 10.1016/j.bioactmat.2021.06.031

80. Yang GH, Kim W, Kim J, Kim G. A skeleton muscle model using GelMA-based cell-aligned bioink processed with an electric-field assisted 3D/4D bioprinting. *Theranostics*. 2021;11(1):48-63. doi: 10.7150/thno.50794

81. Kim D, Hwangbo H, Kim G. Engineered myoblast-laden collagen filaments fabricated using a submerged bioprinting process to obtain efficient myogenic activities. *Biomacromolecules*. 2021;22(12):5042-5051. doi: 10.1021/acs.biomac.1c01006

82. Li S, Dan X, Chen H, *et al*. Developing fibrin-based biomaterials/scaffolds in tissue engineering. *Bioact Mater*. 2024;40:597-623. doi: 10.1016/j.bioactmat.2024.08.006

83. Xuan Z, Peng Q, Larsen T, *et al*. Tailoring hydrogel composition and stiffness to control smooth muscle cell differentiation in bioprinted constructs. *Tissue Eng Regen Med*. 2023;20(2):199-212. doi: 10.1007/s13770-022-00500-1

84. Lou H, Lu H, Zhang S, *et al*. Highly aligned myotubes formation of piscine satellite cells in 3D fibrin hydrogels of cultured meat. *Int J Biol Macromol*. 2024;282:136879. doi: 10.1016/j.ijbiomac.2024.136879

85. Liu J, Song Q, Yin W, *et al*. Bioactive scaffolds for tissue engineering: a review of decellularized extracellular matrix applications and innovations. *Exploration*. 2025;5(1):20230078. doi: 10.1002/EXP.20230078

86. Askari M, Naniz MA, Kouhi M, Saberi A, Zolfagharian A, Bodaghi M. Recent progress in extrusion 3D bioprinting of hydrogel biomaterials for tissue regeneration: a comprehensive review with focus on advanced fabrication techniques. *Biomater Sci*. 2021;9(3):535-573. doi: 10.1039/D0BM00973C

87. Saldin LT, Cramer MC, Velankar SS, White LJ, Badylak SF. Extracellular matrix hydrogels from decellularized tissues: structure and function. *Acta Biomater*. 2017;49(1):1-15. doi: 10.1016/j.actbio.2016.11.068

88. Zhang H, Wang Y, Zheng Z, *et al*. Strategies for improving the 3D printability of decellularized extracellular matrix bioink. *Theranostics*. 2023;13(8):2562-2587. doi: 10.7150/thno.81785

89. Marzi J, Fuhrmann E, Brauchle E, *et al*. Non-invasive three-dimensional cell analysis in bioinks by Raman imaging. *ACS Appl Mater Interfaces*. 2022;14(27):30455-30465. doi: 10.1021/acsami.1c24463

90. Kalva SN, Zakaria Y, Velasquez CA, Koç M. Tailoring the mechanical and degradation properties of 3DP PLA/PCL scaffolds for biomedical applications. *Rev Adv Mater Sci*. 2025;64(1):12. doi: 10.1515/rams-2025-0098

91. Dias JR, Sousa A, Augusto A, Bártolo PJ, Granja PL. Electrospun polycaprolactone (PCL) degradation: an in vitro and in vivo study. *Polymers Basel*. 2022;14(16):3397. doi: 10.3390/polym14163397

92. Hakim Khalili M, Zhang R, Wilson S, Goel S, Impey SA, Aria AI. Additive manufacturing and physicomechanical characteristics of PEGDA hydrogels: recent advances and perspective for tissue engineering. *Polymers Basel*. 2023;15(10):2341. doi: 10.3390/polym15102341

93. Yuan Z, Bai X, Li S, *et al*. Multimaterial and multidimensional bioprinting in regenerative medicine: advances, limitations, and future directions. *Adv Healthc Mater*. 2025 14(18):e2500475. doi: 10.1002/adhm.202500475

94. Sung TC, Wang T, Liu Q, *et al*. Cell-binding peptides on the material surface guide stem cell fate of adhesion, proliferation and differentiation. *J Mater Chem B*. 2023; 11(7):1389-1415. doi: 10.1039/D2TB02601E

95. Cámara-Torres M, Sinha R, Scopece P, *et al*. Tuning cell behavior on 3D scaffolds fabricated by atmospheric plasma-assisted additive manufacturing. *ACS Appl Mater Interfaces*. 2021;13(3):3631-3644. doi: 10.1021/acsami.0c19687

96. Péter B, Boldizsár I, Kovács GM, *et al*. Natural compounds as target biomolecules in cellular adhesion and migration: from biomolecular stimulation to label-free discovery and bioactivity-based isolation. *Biomedicines*. 2021;9(12):1781. doi: 10.3390/biomedicines9121781

97. Bramhe P, Rarokar N, Kumbhalkar R, Saoji S, Khedekar P. Natural and synthetic polymeric hydrogel: a bioink for 3D bioprinting of tissue models. *J Drug Deliv Sci Technol*. 2024;101:106204. doi: 10.1016/j.jddst.2024.106204

98. Khoeini R, Nosrati H, Akbarzadeh A, *et al*. Natural and synthetic bioinks for 3D bioprinting. *Adv NanoBiomed Res*. 2021;1(8):2000097. doi: 10.1002/anbr.202000097

99. Kumar S, Tharayil A, Thomas S. 3D bioprinting of nature-inspired hydrogel inks based on synthetic polymers. *ACS Appl Polym Mater*. 2021;3(8):3685-3701. doi: 10.1021/acsapm.1c00567

100. Fatimi A, Okoro OV, Podstawczyk D, Siminska-Stanny J, Shavandi A. Natural hydrogel-based bio-inks for 3D bioprinting in tissue engineering: a review. *Gels Basel*. 2022;8(3):179. doi: 10.3390/gels8030179

101. Kumar S. Synthetic polymer-derived single-network inks/bioinks for extrusion-based 3D printing towards bioapplications. *Mater Adv*. 2021;2(21):6928-6941. doi: 10.1039/D1MA00525A

102. Satchanska G, Davidova S, Petrov PD. Natural and synthetic polymers for biomedical and environmental applications. *Polymers Basel*. 2024;16(8):1159. doi: 10.3390/polym16081159

103. Reddy MSB, Ponnamma D, Choudhary R, Sadasivuni KK. A comparative review of natural and synthetic biopolymer composite scaffolds. *Polymers Basel*. 2021;13(7):1105. doi: 10.3390/polym13071105

104. Serna JA, Rueda-Gensini L, Céspedes-Valenzuela DN, Cifuentes J, Cruz JC, Muñoz-Camargo C. Recent advances on stimuli-responsive hydrogels based on tissue-derived ECMs and their components: towards improving functionality for tissue engineering and controlled drug delivery. *Polymers Basel*. 2021;13(19):3263. doi: 10.3390/polym13193263

105. Tripathi S, Dash M, Chakraborty R, *et al*. Engineering considerations in the design of tissue specific bioink for 3D bioprinting applications. *Biomater Sci*. 2025;13(1):93-129. doi: 10.1039/D4BM01192A

106. Lee G, Kim YH, Kim D, Lee DH, Bhang SH, Lee K. PCL-fibrin-alginate hydrogel based cell co-culture system for improving angiogenesis and immune modulation in limb ischemia. *Colloid Surface B*. 2025;250:114553. doi: 10.1016/j.colsurfb.2025.114553

107. Grzelak A, Hnydka A, Higuchi J, *et al*. Recent achievements in the development of biomaterials improved with platelet concentrates for soft and hard tissue engineering applications. *Int J Mol Sci*. 2024;25(3):1525. doi: 10.3390/ijms25031525

108. Xiang Y, Gao Y, Cheng Q, *et al*. Recombinant collagen coating 3D printed PEGDA hydrogel tube loading with differentiable BMSCs to repair bile duct injury. *Colloid Surface B*. 2024;241:114064. doi: 10.1016/j.colsurfb.2024.114064

109. Bartholomew K, Mahapatro A. Rheological characterization and print quality studies of gelatin/collagen I/ PEGDA hydrogels. *Int J Polym Mater Polym Biomater*. 2025;74(11):975-986. doi: 10.1080/00914037.2024.2391964

110. Lee J, Lee H, Jin EJ, Ryu D, Kim GH. 3D bioprinting using a new photo-crosslinking method for muscle tissue restoration. *NPJ Regen Med*. 2023;8(1):18. doi: 10.1038/s41536-023-00292-5

111. Altunbek M, Afghah F, Caliskan OS, Yoo JJ, Koc B. Design and bioprinting for tissue interfaces. *Biofabrication*. 2023;15(2):022002. doi: 10.1088/1758-5090/acb73d

112. Heo G, Kim W, Kim G. Comb-assisted 3D bioprinting for highly aligned 3D muscle bioconstructs with enhanced cellular mechanotransduction. *Virtual Phys Prototyp*. 2025;20(1):e2499440. doi: 10.1080/17452759.2025.2499440

113. Zhu Y, Yu X, Liu H, *et al*. Strategies of functionalized GelMA-based bioinks for bone regeneration: recent advances and future perspectives. *Bioact Mater*. 2024;38:346-373. doi: 10.1016/j.bioactmat.2024.04.032

114. Luo W, Zhang H, Wan R, *et al*. Biomaterials-based technologies in skeletal muscle tissue engineering. *Adv Healthc Mater*. 2024;13(18):2304196. doi: 10.1002/adhm.202304196

115. Poerio A, Mashanov V, Lai D, *et al*. Towards innervation of bioengineered muscle constructs: development of a sustained neurotrophic factor delivery and release system. *Bioprinting*. 2022;27:e00220. doi: 10.1016/j.bprint.2022.e00220

116. Sueters J, van Heiningen R, de Vries R, Guler Z, Huirne J, Smit T. Advances in tissue engineering of peripheral nerve and tissue innervation – a systematic review. *J Tissue Eng*. 2025;16:20417314251316918. doi: 10.1177/20417314251316918

117. P. Quint J, Samandari M, Abbasi L, *et al*. Nanoengineered myogenic scaffolds for skeletal muscle tissue engineering. *Nanoscale*. 2022;14(3):797-814. doi: 10.1039/D1NR06143G

118. Quint JP, Mostafavi A, Endo Y, *et al*. In vivo printing of nanoenabled scaffolds for the treatment of skeletal muscle injuries. *Adv Healthc Mater*. 2021;10(10):e2002152. doi: 10.1002/adhm.202002152

119. Huang B. Carbon nanotubes and their polymeric composites: the applications in tissue engineering. *Biomanuf Rev*. 2020;5(1):3. doi: 10.1007/s40898-020-00009-x

120. Kandhola G, Park S, Lim JW, *et al*. Nanomaterial-based scaffolds for tissue engineering applications: a review on graphene, carbon nanotubes and nanocellulose. *Tissue Eng Regen Med*. 2023;20(3):411-433. doi: 10.1007/s13770-023-00530-3

121. Boularaoui S, Shanti A, Lanotte M, *et al*. Nanocomposite conductive bioinks based on low-concentration GelMA and MXene nanosheets/gold nanoparticles providing enhanced printability of functional skeletal muscle tissues. *ACS Biomater Sci Eng*. 2021;7(12):5810-5822. doi: 10.1021/acsbiomaterials.1c01193

122. Ghaziof S, Shojaei S, Mehdikhani M, Khodaei M, Jafari Nodoushan M. Electro-conductive 3D printed polycaprolactone/gold nanoparticles nanocomposite scaffolds for myocardial tissue engineering. *J Mech Behav Biomed*. 2022;132:105271. doi: 10.1016/j.jmbbm.2022.105271

123. Aparicio-Collado JL, García-San-Martín N, Molina-Mateo J, *et al*. Electroactive calcium-alginate/polycaprolactone/reduced graphene oxide nanohybrid hydrogels for skeletal muscle tissue engineering. *Colloid Surface B*. 2022;214:112455. doi: 10.1016/j.colsurfb.2022.112455

124. Jo SB, Erdenebileg U, Dashnyam K, *et al*. Nano-graphene oxide/polyurethane nanofibers: mechanically flexible and myogenic stimulating matrix for skeletal tissue engineering. *J Tissue Eng*. 2020;11:2041731419900424. doi: 10.1177/2041731419900424

125. Han S, Cruz SH, Park S, Shin SR. Nano-biomaterials and advanced fabrication techniques for engineering skeletal muscle tissue constructs in regenerative medicine. *Nano Converg*. 2023;10(1):48. doi: 10.1186/s40580-023-00398-y

126. Jo HJ, Kang MS, Heo HJ, *et al*. Skeletal muscle regeneration with 3D bioprinted hyaluronate/gelatin hydrogels incorporating MXene nanoparticles. *Int J Biol Macromol*. 2024;265:130696. doi: 10.1016/j.ijbiomac.2024.130696

127. Liu L, Wang C, Wu Z, Xing Y. Ultralow-voltage-drivable artificial muscles based on a 3D structure MXene-PEDOT:PSS/AgNWs electrode. *ACS Appl Mater Interfaces*. 2022;14(16):18150-18158. doi: 10.1021/acsami.2c00760

128. Oldroyd P, Oldroyd S, Meng M, *et al*. Stretchable device for simultaneous measurements of contractility and electrophysiology of neuromuscular tissue in the gastrointestinal tract. *Adv Mater*. 2024;36(19):2312735. doi: 10.1002/adma.202312735

129. Nain A, Chakraborty S, Jain N, *et al*. 4D hydrogels: fabrication strategies, stimulation mechanisms, and biomedical applications. *Biomater Sci*. 2024;12(13):3249-3272. doi: 10.1039/D3BM02044D

130. Arif ZU, Khalid MY, Zolfagharian A, Bodaghi M. 4D bioprinting of smart polymers for biomedical applications: Recent progress, challenges, and future perspectives. *React Funct Polym*. 2022;179:105374. doi: 10.1016/j.reactfunctpolym.2022.105374

131. Wang Y, Cui H, Esworthy T, Mei D, Wang Y, Zhang LG. Emerging 4D printing strategies for next-generation

tissue regeneration and medical devices. *Adv Mater*. 2022;34(20):2109198.
doi: 10.1002/adma.202109198

132. Fuentes J, Guix M, Cenev ZM, *et al*. Ferrofluid-based bioink for 3D printed skeletal muscle tissues with enhanced force and magnetic response. *Adv Mater Interfaces*. 2025;12(13):2400824.
doi: 10.1002/admi.202400824

133. Tuftee C, Alsberg E, Ozbolat IT, Rizwan M. Emerging granular hydrogel bioinks to improve biological function in bioprinted constructs. *Trends Biotechnol*. 2024;42(3):339-352.
doi: 10.1016/j.tibtech.2023.09.007

134. Zhang S, Li G, Man J, *et al*. Fabrication of microspheres from high-viscosity bioink using a novel microfluidic-based 3D bioprinting nozzle. *Micromachines Basel*. 2020;11(7):681.
doi: 10.3390/mi11070681

135. Yang J, Zhang X, Xie J, *et al*. Porous mullite ceramics with hierarchical pores constructed via vat photopolymerization of multi-phase pickering emulsion. *Addit Manuf*. 2024;79:103943.
doi: 10.1016/j.addma.2023.103943

136. Tuladhar S, Clark S, Habib A. Tuning shear thinning factors of 3D bio-printable hydrogels using short fiber. *Materials*. 2023;16(2):572.
doi: 10.3390/ma16020572

137. Sánchez-Sánchez R, Rodríguez-Rego JM, Macías-García A, Mendoza-Cerezo L, Díaz-Parralejo A. Relationship between shear-thinning rheological properties of bioinks and bioprinting parameters. *Int J Bioprinting*. 2023;9(2):687.
doi: 10.18063/ijb.687

138. de Barros NR, Darabi MA, Ma X, *et al*. Enhanced maturation of 3D bioprinted skeletal muscle tissue constructs encapsulating soluble factor-releasing microparticles. *Macromol Biosci*. 2023;23(12):e2300276.
doi: 10.1002/mabi.202300276

139. Cho S, Jang J. Recent trends in biofabrication technologies for studying skeletal muscle tissue-related diseases. *Front Bioeng Biotechnol*. 2021;9.
doi: 10.3389/fbioe.2021.782333

140. Fornetti E, De Paolis F, Fuoco C, *et al*. A novel extrusion-based 3D bioprinting system for skeletal muscle tissue engineering. *Biofabrication*. 2023;15(2):025009.
doi: 10.1088/1758-5090/acb573

141. Zhan Y, Jiang W, Liu Z, Wang Z, Guo K, Sun J. Utilizing bioprinting to engineer spatially organized tissues from the bottom-up. *Stem Cell Res Ther*. 2024;15(1):101.
doi: 10.1186/s13287-024-03712-5

142. Zoghi S. Advancements in tissue engineering: a review of bioprinting techniques, scaffolds, and bioinks. *Biomed Eng Comput Biol*. 2024;15:11795972241288099.
doi: 10.1177/11795972241288099

143. Sonaye SY, Ertugral EG, Kothapalli CR, Sikder P. Extrusion 3D (bio)printing of alginate-gelatin-based composite scaffolds for skeletal muscle tissue engineering. *Materials*. 2022;15(22):7945.
doi: 10.3390/ma15227945

144. Malekpour A, Chen X. Printability and cell viability in extrusion-based bioprinting from experimental, computational, and machine learning views. *J Funct Biomater*. 2022;13(2):40.
doi: 10.3390/jfb13020040

145. McCauley PJ, Fromen CA, Bayles AV. Cell viability in extrusion bioprinting: The impact of process parameters, bioink rheology, and cell mechanics. *Rheol Acta*. 2025.
doi: 10.1007/s00397-025-01504-z

146. Betancourt N, Chen X. Review of extrusion-based multi-material bioprinting processes. *Bioprinting*. 2022;25:e00189.
doi: 10.1016/j.bprint.2021.e00189

147. Rossi A, Pescara T, Gambelli AM, *et al*. Biomaterials for extrusion-based bioprinting and biomedical applications. *Front Bioeng Biotechnol*. 2024;12:1393641.
doi: 10.3389/fbioe.2024.1393641

148. Müller SJ, Fabry B, Gekle S. Predicting cell stress and strain during extrusion bioprinting. *Phys Rev Appl*. 2023;19(6):064061.
doi: 10.1103/PhysRevApplied.19.064061

149. Lombardi L, Scalzone A, Ausilio C, Gentile P, Tammaro D. Optimizing nozzle design in extrusion-based 3D bioprinting to minimize mechanical stress and enhance cell viability. *Int J Bioprinting*. 2025:e025190182.
doi: 10.36922/IJB025190182

150. Reina-Romo E, Mandal S, Amorim P, Bloemen V, Ferraris E, Geris L. Towards the experimentally-informed in silico nozzle design optimization for extrusion-based bioprinting of shear-thinning hydrogels. *Front Bioeng Biotechnol*. 2021;9:701778.
doi: 10.3389/fbioe.2021.701778

151. Rasouli R, Sweeney C, Frampton JP. Heterogeneous and composite bioinks for 3D-bioprinting of complex tissue. *Biomed Mater Devices*. 2025;3(1):108-126.
doi: 10.1007/s44174-024-00171-7

152. Budharaju H, Sundaramurthi D, Sethuraman S. Embedded 3D bioprinting – an emerging strategy to fabricate biomimetic & large vascularized tissue constructs. *Bioact Mater*. 2024;32:356-384.
doi: 10.1016/j.bioactmat.2023.10.012

153. Kumar P, Ebbens S, Zhao X. Inkjet printing of mammalian cells – theory and applications. *Bioprinting*. 2021;23:e00157.
doi: 10.1016/j.bprint.2021.e00157

154. Guida L, Cavallaro M, Levi M. Advancements in high-resolution 3D bioprinting: Exploring technological trends, bioinks and achieved resolutions. *Bioprinting*. 2024;44:e00376.
doi: 10.1016/j.bprint.2024.e00376

155. Huang J, Zhou G, Jiang Q, Li L. *In situ* 3D bioprinting: the future of regenerative medicine. *Fundam Res*. 2025. doi: 10.1016/j.fmre.2025.06.004

156. Ng WL, Shkolnikov V. Optimizing cell deposition for inkjet-based bioprinting. *Int J Bioprinting*. 2024;10(2):2135. doi: 10.36922/ijb.2135

157. Cheng C, Williamson EJ, Chiu GTC, Han B. Engineering biomaterials by inkjet printing of hydrogels with functional particulates. *Med-X*. 2024;2(1):9. doi: 10.1007/s44258-024-00024-4

158. Xu HQ, Liu JC, Zhang ZY, Xu CX. A review on cell damage, viability, and functionality during 3D bioprinting. *Mil Med Res* 2022;9(1):70. doi: 10.1186/s40779-022-00429-5

159. Ghosh E, Rego GP, Ghosh RN, *et al*. Advances in in situ bioprinting: a focus on extrusion and inkjet-based bioprinting techniques. *Regen Eng Transl Med*. 2025. doi: 10.1007/s40883-025-00420-1

160. Chang J, Sun X. Laser-induced forward transfer based laser bioprinting in biomedical applications. *Front Bioeng Biotechnol*. 2023;11:1255782. doi: 10.3389/fbioe.2023.1255782

161. Kryou C, Zergioti I. Laser-induced forward transfer on regenerative medicine applications. *Biomed Mater Devices*. 2023;1(1):5-20. doi: 10.1007/s44174-022-00040-1

162. Leberfinger AN, Dinda S, Wu Y, *et al*. Bioprinting functional tissues. *Acta Biomater*. 2019;95(1):32-49. doi: 10.1016/j.actbio.2019.01.009

163. Bosmans C, Ginés Rodriguez N, Karperien M, *et al*. Towards single-cell bioprinting: micropatterning tools for organ-on-chip development. *Trends Biotechnol*. 2024;42(6):739-759. doi: 10.1016/j.tibtech.2023.11.014

164. Sun J, Gong Y, He Y, *et al*. Process optimization for coaxial extrusion-based bioprinting: a comprehensive analysis of material behavior, structural precision, and cell viability. *Addit Manuf*. 2025;100:104682. doi: 10.1016/j.addma.2025.104682

165. Mohan TS, Datta P, Nesaei S, Ozbolat V, Ozbolat IT. 3D coaxial bioprinting: process mechanisms, bioinks and applications. *Prog Biomed Eng (Bristol)*. 2022;4(2):022003. doi: 10.1088/2516-1091/ac631c

166. Eghosasere E, Osasumwen E, Emmanuella O. 3D bioprinting in tissue engineering: Advancements, challenges, and pathways to clinical translation. *JSM Regen Med Bioeng*. 2025;7(1):1-15. doi: 10.47739/2379-0490/1023

167. Volpi M, Paradiso A, Walejewska E, Gargioli C, Costantini M, Swieszkowski W. Automated microfluidics-assisted hydrogel-based wet-spinning for the biofabrication of biomimetic engineered myotendinous junction. *Adv Healthc Mater*. 2024;13(32):e2402075. doi: 10.1002/adhm.202402075

168. Mohammadi S, Cidonio G. Unravelling hierarchical patterning of biomaterial inks with 3D microfluidic-assisted spinning: a paradigm shift in bioprinting technologies. *Front Biomater Sci*. 2023;2:1279061. doi: 10.3389/fbiom.2023.1279061

169. Serpe F, Casciola CM, Ruocco G, Cidonio G, Scognamiglio C. Microfluidic fiber spinning for 3D bioprinting: harnessing microchannels to build macrotissues. *Int J Bioprinting*. 2024;10(1):1404. doi: 10.36922/ijb.1404

170. Hosseinabadi HG, Dogan E, Miri AK, Ionov L. Digital light processing bioprinting advances for micro-tissue models. *ACS Biomater Sci Eng*. 2022;8(4):1381-1395. doi: 10.1021/acsbiomaterials.1c01509

171. Jeong YG, Yoo JJ, Lee SJ, Kim MS. 3D digital light process bioprinting: cutting-edge platforms for resolution of organ fabrication. *Mater Today Bio*. 2024;29:101284. doi: 10.1016/j.mtbio.2024.101284

172. Seo JW, Kim GM, Choi Y, Cha JM, Bae H. Improving printability of digital-light-processing 3D bioprinting via photoabsorber pigment adjustment. *Int J Mol Sci*. 2022;23(10):5428. doi: 10.3390/ijms23105428

173. Karakaidos P, Kryou C, Simigdala N, Klinakis A, Zergioti I. Laser bioprinting of cells using UV and visible wavelengths: a comparative DNA damage study. *Bioengineering*. 2022;9(8):378. doi: 10.3390/bioengineering9080378

174. Silva C, Cortés-Rodriguez CJ, Hazur J, Reakasame S, Boccaccini AR. Rational design of a triple-layered coaxial extruder system: in silico and in vitro evaluations directed toward optimizing cell viability. *Int J Bioprinting*. 2020;6(4):282. doi: 10.18063/ijb.v6i4.282

175. Zhang P, Liu C, Modavi C, Abate A, Chen H. Printhead on a chip: empowering droplet-based bioprinting with microfluidics. *Trends Biotechnol*. 2024;42(3):353-368. doi: 10.1016/j.tibtech.2023.09.001

176. Duong VT, Lin CC. Digital light processing 3D bioprinting of gelatin-norbornene hydrogel for enhanced vascularization. *Macromol Biosci*. 2023;23(12):e2300213. doi: 10.1002/mabi.202300213

177. Cao T, Warren CR. From 2D myotube cultures to 3D engineered skeletal muscle constructs: a comprehensive review of in vitro skeletal muscle models and disease modeling applications. *Cells Basel*. 2025;14(12):882. doi: 10.3390/cells14120882

178. Kim J, Lee H, Lee G, Ryu D, Kim G. Fabrication of fully aligned self-assembled cell-laden collagen filaments for tissue engineering *via* a hybrid bioprinting process. *Bioact Mater*. 2024;36:14-29. doi: 10.1016/j.bioactmat.2024.02.020

179. Hwangbo H, Koo Y, Nacionales F, Kim J, Chae S, Kim GH. Stimulus-assisted in situ bioprinting: advancing direct bench-to-bedside delivery. *Trends Biotechnol*. 2025;43(5):1015-1030. doi: 10.1016/j.tibtech.2024.11.001

180. Hwangbo H, Chae S, Ryu D, Kim G. *In situ* magnetic-field-assisted bioprinting process using magnetorheological bioink to obtain engineered muscle constructs. *Bioact Mater*. 2025;45:417-433. doi: 10.1016/j.bioactmat.2024.11.035

181. Gao H, Xiao J, Wei Y, Wang H, Wan H, Liu S. Regulation of myogenic differentiation by topologically microgrooved surfaces for skeletal muscle tissue engineering. *ACS Omega*. 2021;6(32):20931-20940. doi: 10.1021/acsomega.1c02347

182. Kamal KY, Othman MA, Kim JH, Lawler JM. Bioreactor development for skeletal muscle hypertrophy and atrophy by manipulating uniaxial cyclic strain: proof of concept. *NPJ Microgravity*. 2024;10(1):62. doi: 10.1038/s41526-023-00320-0

183. Borisov V, Gili Sole L, Reid G, *et al*. Upscaled skeletal muscle engineered tissue with in vivo vascularization and innervation potential. *Bioengineering*. 2023;10(7):800. doi: 10.3390/bioengineering10070800

184. Wang X, Dong W, Dong H, *et al*. Bioprinting of wearable sensors, brain-machine interfaces, and exoskeleton robots. *Int J Bioprinting*. 2024;10(6):3590. doi: 10.36922/ijb.3590

185. Singh S, Choudhury D, Yu F, Mironov V, Naing MW. In situ bioprinting - bioprinting from benchside to bedside? *Acta Biomater*. 2020;101(1):14-25. doi: 10.1016/j.actbio.2019.08.045

186. Gorbenko N, Vaccaro JC, Fagan R, *et al*. Perfusion bioreactor conditioning of small-diameter plant-based vascular grafts. *Tissue Eng Regen Med*. 2024;21(8):1189-1201. doi: 10.1007/s13770-024-00670-0

187. Ng WL, An J, Chua CK. Process, material, and regulatory considerations for 3D printed medical devices and tissue constructs. *Engineering*. 2024;36(5):146-166. doi: 10.1016/j.eng.2024.01.028

188. Mladenovska T, Choong PF, Wallace GG, O'Connell CD. The regulatory challenge of 3D bioprinting. *Regen Med*. 2023;18(8):659-674. doi: 10.2217/rme-2022-0194

189. Yoshida A, Baba K, Takahashi H, Nagase K, Shimizu T. One-step fabrication of 3D-aligned human skeletal muscle tissue and measurement of contractile force for preclinical drug testing. *Mater Today Bio*. 2025;31:101456. doi: 10.1016/j.mtbio.2025.101456

190. Gao L, Li L, Wu W, *et al*. 3D-bioprinted in vitro skeletal muscle with pennate fiber architecture to enhance contractile function. *Int J Bioprinting*. 2024;10(6):4371. doi: 10.36922/ijb.4371

191. Carraro E, Rossi L, Maghin E, Canton M, Piccoli M. 3D in vitro models of pathological skeletal muscle: which cells and scaffolds to elect? *Front Bioeng Biotechnol*. 2022;10:941623. doi: 10.3389/fbioe.2022.941623

192. Mazzoldi EL, Gaudenzi G, Ginestra PS, Ceretti E, Giliani SC. Evaluating cells metabolic activity of bioinks for bioprinting: the role of cell-laden hydrogels and 3D printing on cell survival. *Front Bioeng Biotechnol*. 2024;12:1450838. doi: 10.3389/fbioe.2024.1450838

193. Pellegrini E, Desando G, Petretta M, *et al*. A 3D collagen-based bioprinted model to study osteosarcoma invasiveness and drug response. *Polymers*. 2022;14(19):4070. doi: 10.3390/polym14194070

194. Avnet S, Pompo GD, Borciani G, Fischetti T, Graziani G, Baldini N. Advantages and limitations of using cell viability assays for 3D bioprinted constructs. *Biomed Mater*. 2024;19(2):025033. doi: 10.1088/1748-605X/ad2556

195. VanGenderen CA, Granet JA, Filippelli RL, Liu Y, Chang NC. Modulating myogenesis: an optimized in vitro assay to pharmacologically influence primary myoblast differentiation. *Curr Protoc*. 2022;2(9):e565. doi: 10.1002/cpz1.565

196. Mueller C, Trujillo-Miranda M, Maier M, Heath DE, O'Connor AJ, Salehi S. Effects of external stimulators on engineered skeletal muscle tissue maturation. *Adv Mater Interfaces*. 2021;8(1):2001167. doi: 10.1002/admi.202001167

197. Vesga-Castro C, Aldazabal J, Vallejo-Illarramendi A, Paredes J. Contractile force assessment methods for in vitro skeletal muscle tissues. Huang CLH, Zaidi M, eds. *Elife*. 2022;11:e77204. doi: 10.7554/eLife.77204

198. Khodabukus A. Tissue-engineered skeletal muscle models to study muscle function, plasticity, and disease. *Front Physiol*. 2021;12:619710. doi: 10.3389/fphys.2021.619710

199. Ebrahimi M, Lad H, Fusto A, *et al*. De novo revertant fiber formation and therapy testing in a 3D culture model of duchenne muscular dystrophy skeletal muscle. *Acta Biomater*. 2021;132(1):227-244. doi: 10.1016/j.actbio.2021.05.020

200. Tejedera-Villafranca A, Montolio M, Ramón-Azcón J, Fernández-Costa JM. Mimicking sarcolemmal damagein vitro: a contractile 3D model of skeletal muscle for drug testing in duchenne muscular dystrophy. *Biofabrication*. 2023;15(4). doi: 10.1088/1758-5090/acfb3d

201. Torres MJ, Zhang X, Slentz DH, *et al*. Chemotherapeutic drug screening in 3D-bioengineered human myobundles provides insight into taxane-induced myotoxicities. *Iscience*. 2022;25(10):105189. doi: 10.1016/j.isci.2022.105189

202. Madden L, Juhas M, Kraus WE, Truskey GA, Bursac N. Bioengineered human myobundles mimic clinical responses of skeletal muscle to drugs. Wagers AJ, ed. *Elife*. 2015;4:e04885. doi: 10.7554/eLife.04885

203. Raman R, Cvetkovic C, Uzel SGM, *et al*. Optogenetic skeletal muscle-powered adaptive biological machines. *Proc Natl Acad Sci U S A*. 2016;113(13):3497-3502. doi: 10.1073/pnas.1516139113

204. Filippi M, Badolato A, Georgopoulou A, *et al*. Bioprinting of piezoresistive organohydrogel networks for advanced real-time mechanosensing in engineered tissue models. *Trends Biotechnol*. 2025:S0167-7799(25)00212-4. doi: 10.1016/j.tibtech.2025.05.026

205. Loi G, Scocozza F, Benedetti L, *et al*. Design, development, and benchmarking of a bioreactor integrated with 3D bioprinting: application to skeletal muscle regeneration. *Bioprinting*. 2024;42:e00352. doi: 10.1016/j.bprint.2024.e00352

206. Rodriguez Ayala A, Christ G, Griffin D. Cell-scale porosity minimizes foreign body reaction and promotes innervated myofiber formation after volumetric muscle loss. *NPJ Regen Med*. 2025;10(1):12. doi: 10.1038/s41536-025-00395-1

207. Niknezhad SV, Mehrali M, Khorasgani FR, *et al*. Enhancing volumetric muscle loss (VML) recovery in a rat model using super durable hydrogels derived from bacteria. *Bioact Mater*. 2024;38:540-558. doi: 10.1016/j.bioactmat.2024.04.006

208. Zhao N, Huang Y, Cheng X, *et al*. A critical size volumetric muscle loss model in mouse masseter with impaired mastication on nutrition. *Cell Proliferat*. 2024; 57(6):e13610. doi: 10.1111/cpr.13610

209. Whitaker R, Sung S, Tylek T, *et al*. Effects of injury size on local and systemic immune cell dynamics in volumetric muscle loss. *NPJ Regen Med*. 2025;10(1):9. doi: 10.1038/s41536-025-00397-z

210. Fischer EO, Tsukerman A, Machour M, *et al*. Bioprinting perfusable and vascularized skeletal muscle flaps for the treatment of volumetric muscle loss. *Adv Healthc Mater*. 2025;14(13):e2404542. doi: 10.1002/adhm.202404542

211. Yamaoka Y, Chan WI, Seno S, Iwamori K, Fukada SI, Matsuda H. Quantifying the recovery process of skeletal muscle on hematoxylin and eosin stained images via learning from label proportion. *Sci Rep*. 2024; 14(1):27044. doi: 10.1038/s41598-024-78433-z

212. Rana SP, Dey M. Detrended fluctuation analysis of gait cycles: a study of neuromuscular and ground force dynamics. *Sensors Basel*. 2025;25(13):4122. doi: 10.3390/s25134122

213. Hoffman DB, Schifino AG, Cooley MA, *et al*. Low intensity, high frequency vibration training to improve musculoskeletal function in a mouse model of volumetric muscle loss. *J Orthop Res*. 2025;43(3):622-631. doi: 10.1002/jor.26023

214. Johnson D, Tobo C, Au J, *et al*. Combined regenerative rehabilitation improves recovery following volumetric muscle loss injury in a rat model. *J Biomed Mater Res B*. 2024;112(7):e35438. doi: 10.1002/jbm.b.35438

215. Mehrotra P, Jablonski J, Toftegaard J, *et al*. Skeletal muscle reprogramming enhances reinnervation after peripheral nerve injury. *Nat Commun*. 2024;15(1):9218. doi: 10.1038/s41467-024-53276-4

216. Hoffman DB, Raymond-Pope CJ, Pritchard EE, *et al*. Differential evaluation of neuromuscular injuries to understand re-innervation at the neuromuscular junction. *Exp Neurol*. 2024;382(1):114996. doi: 10.1016/j.expneurol.2024.114996

217. Fabre P, Molina T, Larose J, *et al*. Bioactive lipid mediator class switching regulates myogenic cell progression and muscle regeneration. *Nat Commun*. 2025;16(1):5578. doi: 10.1038/s41467-025-60586-8

218. Zhu C, Sklyar K, Karvar M, Endo Y, Sinha I. Scaffold tissue engineering strategies for volumetric muscle loss. *PAR*. 2023;10:58. doi: 10.20517/2347-9264.2022.89

219. Bülow A, Schäfer B, Beier JP. Three-dimensional bioprinting in soft tissue engineering for plastic and reconstructive surgery. *Bioengineering*. 2023;10(10):1232. doi: 10.3390/bioengineering10101232

220. Lee MC, Jodat YA, Endo Y, *et al*. Engineering large-scale hiPSC-derived vessel-integrated muscle-like lattices for enhanced volumetric muscle regeneration. *Trends Biotechnol*. 2024;42(12):1715-1744. doi: 10.1016/j.tibtech.2024.08.001

221. Zhou Z, Liu J, Xiong T, Liu Y, Tuan RS, Li ZA. Engineering innervated musculoskeletal tissues for regenerative orthopedics and disease modeling. *Small*. 2024;20(23):2310614. doi: 10.1002/smll.202310614

222. Sicherer ST, Venkatarama RS, Grasman JM. Recent trends in injury models to study skeletal muscle regeneration and repair. *Bioengineering*. 2020;7(3):76. doi: 10.3390/bioengineering7030076

223. Fakhr MJ, Kavakebian F, Ababzadeh S, Rezapour A. Challenges and advances in peripheral nerve tissue engineering critical factors affecting nerve regeneration. *J Tissue Eng Regen Med*. 2024;1:8868411. doi: 10.1155/2024/8868411

224. Garg K, Brockhouse J, McAndrew CM, *et al*. Regenerative rehabilitation: navigating the gap between preclinical

promises and clinical realities for treating trauma-induced volumetric muscle loss. *J Physiol*. 2025.
doi: 10.1113/JP286551

225. Jo B, Motoi K, Morimoto Y, Takeuchi S. Dynamic and static workout of in vitro skeletal muscle tissue through a weight training device. *Adv Healthc Mater*. 2024.
doi: 10.1002/adhm.202401844

226. Vesga-Castro C, Mosqueira-Martín L, Ubiria-Urkola P, *et al*. Development of an in vitro platform for the analysis of contractile and calcium dynamics in single human myotubes. *Lab Chip*. 2024;24(20):4741-4754.
doi: 10.1039/D3LC00442B

227. Rousseau E, Raman R, Tamir T, *et al*. Actuated tissue engineered muscle grafts restore functional mobility after volumetric muscle loss. *Biomaterials*. 2023;302(1): 122317.
doi: 10.1016/j.biomaterials.2023.122317

228. De Paolis F, Testa S, Guarnaccia G, *et al*. Long-term longitudinal study on swine VML model. *Biol Direct*. 2023;18(1):42.
doi: 10.1186/s13062-023-00399-1

229. Costantini M, Testa S, Fornetti E, *et al*. Biofabricating murine and human myo-substitutes for rapid volumetric muscle loss restoration. *EMBO Mol Med*. 2021;13(3): e12778.
doi: 10.15252/emmm.202012778

230. Humphrey JD. Constrained mixture models of soft tissue growth and remodeling – twenty years after. *J Elasticity*. 2021;145(1):49-75.
doi: 10.1007/s10659-020-09809-1

231. Roche CD, Sharma P, Ashton AW, Jackson C, Xue M, Gentile C. Printability, durability, contractility and vascular network formation in 3D bioprinted cardiac endothelial cells using alginate–gelatin hydrogels. *Front Bioeng Biotechnol*. 2021;9.
doi: 10.3389/fbioe.2021.636257

232. Kamaraj M, Moghimi N, Joshi A, *et al*. Recent advances in handheld and robotic bioprinting approach for tissue engineering. *Adv Mater Technol*. 2025;10(15):2500206.
doi: 10.1002/admt.202500206

233. Freeman S, Calabro S, Williams R, Jin S, Ye K. Bioink formulation and machine learning-empowered bioprinting optimization. *Front Bioeng Biotechnol*. 2022;10.
doi: 10.3389/fbioe.2022.913579

234. Chen H, Liu Y, Balabani S, Hirayama R, Huang J. Machine learning in predicting printable biomaterial formulations for direct ink writing. *Research*. 2023;0197.
doi: 10.34133/research.0197

235. Zhang Z, Zhou X, Fang Y, Xiong Z, Zhang T. AI-driven 3D bioprinting for regenerative medicine: from bench to bedside. *Bioact Mater*. 2025;45:201-230.
doi: 10.1016/j.bioactmat.2024.11.021

236. Kim MH, Singh YP, Celik N, *et al*. High-throughput bioprinting of spheroids for scalable tissue fabrication. *Nat Commun*. 2024;15(1):10083.
doi: 10.1038/s41467-024-54504-7

237. Jung H, Lee H, Shin M, Son D. Adhesive bioelectronics for closed-loop therapy. *Med-X*. 2025;3(1):11.
doi: 10.1007/s44258-025-00055-5

238. Boufidis D, Garg R, Angelopoulos E, Cullen DK, Vitale F. Bio-inspired electronics: Soft, biohybrid, and "living" neural interfaces. *Nat Commun*. 2025;16(1):1861.
doi: 10.1038/s41467-025-57016-0

239. Ploeger H, Gonzalez-Molina J. Designing biomimetic protocells and prototissues as smart biomaterials. *Cell Biomater*. 2025;(in press)
doi: 10.1016/j.celbio.2025.100097

240. Briones Y, Pascua B, Tiangco N, Crisostomo I, Casiguran S, Remenyi R. Assessing the landscape of clinical and observational trials involving bioprinting: a scoping review. *3D Print Med*. 2025;11(1):5.
doi: 10.1186/s41205-025-00253-2