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Bridging biofabrication and bioreactor-based maturation in muscle tissue engineering / Van Meerssche, J., Kalogeropoulou, M., Shajahan, A.B., Gabetti, S., Massai, D., Porsborg, S.R., Pien, N.. - In: FRONTIERS IN BIOENGINEERING AND BIOTECHNOLOGY. - ISSN 2296-4185. - ELETTRONICO. - 14:(2026), pp. 1-46. [10.3389/fbioe.2026.1934389]

Availability:
This version is available at: 11583/3018192 since: 2026-11-20T11:46:42Z

Publisher:
Frontiers

Published
DOI:10.3389/fbioe.2026.1934389

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27 November 2026

frontiers
in Bioengineering and
Biotechnology

TYPE Review
PUBLISHED 13 November 2026
DOI 10.3389/fbioe.2026.1934389

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RECEIVED 10 July 2026
REVISED 08 September 2026
ACCEPTED 20 September 2026
PUBLISHED 13 November 2026

CITATION
Van Meerssche J, Kalogeropoulou M, Shajahan AB, Gabetti S, Massai D, Porsborg SR and Pien N (2026) Bridging biofabrication and bioreactor-based maturation in muscle tissue engineering. *Front. Bioeng. Biotechnol.* 14:1934389. doi: 10.3389/fbioe.2026.1934389

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Bridging biofabrication and bioreactor-based maturation in muscle tissue engineering

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Engineering functional skeletal, cardiac, and smooth muscle tissues requires the integration of advanced biofabrication technologies with physiologically relevant maturation strategies. However, biofabrication and bioreactor development have often been considered separately, leaving the benefits of integrated construct-bioreactor design incompletely understood. This review provides an integrated overview of biofabrication and bioreactor-based maturation strategies for muscle tissue engineering. We discuss how muscle-specific requirements influence biomaterial selection, construct architecture, and stimulation approaches, and summarize recent advances in biofabrication and mechanical, electrical, perfusion, and multimodal bioreactor systems. Finally, we highlight key challenges and opportunities in construct-bioreactor co-design and argue that integrating biofabrication with tissue-specific bioreactor conditioning will be critical for translating structural complexity into functional muscle tissues.

KEYWORDS
biofabrication, bioprinting, bioreactor, cardiac muscle, muscle tissue engineering, skeletal muscle, smooth muscle, tissue maturation

1 Introduction

The engineering of functional skeletal, cardiac, and smooth muscle tissues has emerged as one of the major challenges in regenerative medicine, with applications ranging from volumetric skeletal muscle loss and myocardial infarction to vascular and gastrointestinal smooth muscle disorders. Despite advances in biomaterials, stem cell biology, and biofabrication technologies, recreating the structural and functional complexity of native muscle tissues remains difficult (Groll et al., 2016; Lim et al., 2020). While all three muscle types share a contractile function, they differ substantially in their hierarchical organization, mechanical environment, electrophysiological behavior, metabolic demand, and regenerative capacity (Baldwin et al., 2024; Al Khafaji et al., 2025; Cordelle et al., 2025). Consequently, successful muscle tissue engineering requires not only precise control over construct architecture but also the application of tissue-specific maturation cues that guide cellular organization and functional development.

Recent advances in biofabrication have enabled the generation of increasingly complex three-dimensional muscle constructs with controlled geometry, spatial cell organization, and biomimetic microarchitectures using extrusion-based and light-based manufacturing technologies (Levato et al., 2020; Lim et al., 2020; Ng et al., 2025; Prados-Martin et al., 2026). At the same time, bioreactor systems have evolved to provide controlled mechanical, electrical, and perfusion-based stimulation that promotes tissue maturation by recreating

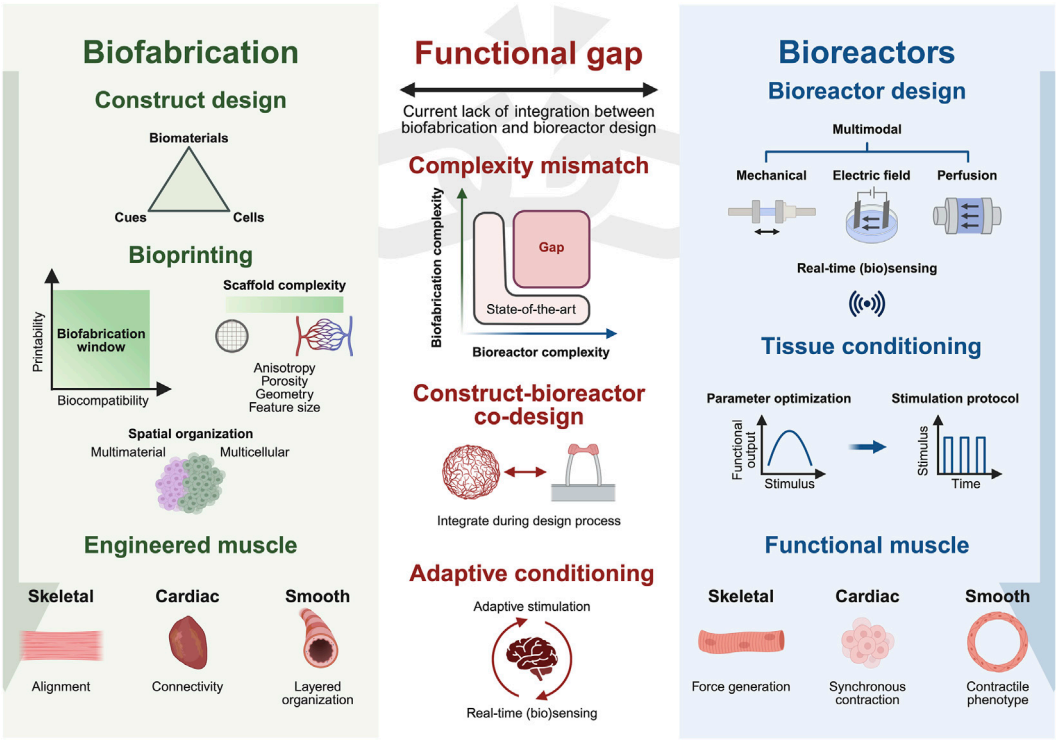

FIGURE 1
Conceptual framework illustrating the functional gap between biofabrication and bioreactor-based maturation for muscle tissue engineering. (Left) Advanced biofabrication strategies generate increasingly complex skeletal, cardiac, and smooth muscle constructs. (Center) A functional gap persists between biofabrication and bioreactor-induced maturation. (Right) Next-generation bioreactors condition muscle tissue to promote tissue maturation and functional development. Created in BioRender.

aspects of the native physiological environment (Paez-Mayorga et al., 2018; Zhang and Habibovic, 2022; Williamson et al., 2024). However, both fields have often progressed independently. Biofabrication has primarily focused on increasing structural complexity, whereas bioreactor development has concentrated on optimizing stimulation protocols for relatively simple constructs. As a result, many engineered tissues exhibit a functional gap between the complexity achieved during fabrication and the level of functional maturation attained during culture (see Figure 1).

In this review, we provide an integrated overview of biofabrication strategies and bioreactor-based maturation approaches for skeletal, cardiac, and smooth muscle tissue engineering. Rather than discussing these technologies independently, we highlight how construct design influences subsequent bioreactor performance and *vice versa*. We identify current challenges, discuss opportunities for construct-bioreactor co-design, and propose integrated workflows that combine advanced biofabrication, adaptive tissue conditioning, and real-time monitoring to accelerate the development of functional and clinically translatable engineered muscle tissues.

2 Tissue engineering aspects of skeletal, cardiac and smooth muscle

The human body contains three major muscle types, skeletal, cardiac, and smooth muscle, each characterized by distinct structural organization, physiological function, regenerative capacity, and maturation requirements. These differences influence the design criteria for engineered muscle constructs and their subsequent maturation strategies.

Recapitulating muscle function and organization *in vitro* poses certain engineering and biological challenges. Engineering functional muscle tissues requires mimicking key structural, cellular, and physicochemical features through biofabrication, in conjunction with the application of biophysical cues that drive tissue development, maintenance, and functional maturation *in vivo*. The structural and functional differences between skeletal, cardiac, and smooth muscle are reflected in their stiffness, force generation, and regenerative capacity, among others (see Table 1). The structural and functional properties of native skeletal, cardiac, and smooth muscle are discussed below as a starting point for the development of functional engineered muscle tissues.

2.1 Skeletal muscle structure and function

Skeletal muscle is responsible for limb control, posture support, and locomotion (see Figure 2) (King et al., 2004). Structurally, skeletal muscle has a hierarchical, anisotropic structure, in which higher-order structures are formed in the order of sarcomeres, myofibrils, myofibers, and fascicles (King et al., 2004). Contraction starts at the neuromuscular junction where motion is achieved via ATP-dependent actin-myosin interactions (Mukund and Subramaniam, 2020). Skeletal muscle tissue is highly vascularized and innervated to support efficient energy production and cellular homeostasis (Mukund and Subramaniam, 2020).

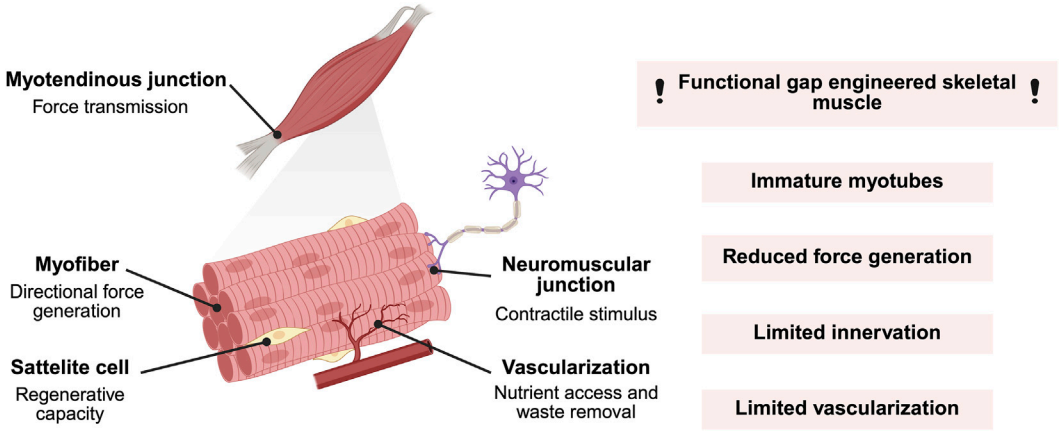

FIGURE 2
Native skeletal muscle structure and key limitations of engineered skeletal muscle tissues. Native skeletal muscle contains anisotropic organized myofibers, neuromuscular junctions, satellite cells, vascular networks, and myotendinous junctions that collectively support force generation, regeneration, and tissue homeostasis. Engineered skeletal muscle tissues frequently exhibit immature myotubes, reduced force generation, limited vascularization, and limited innervation. Created in BioRender.

TABLE 1 **Properties overview of mature, healthy *in vivo* skeletal, cardiac, and smooth muscle.**

Property	Skeletal muscle	Cardiac muscle	Smooth muscle
Primary function	Movement	Blood circulation	Control of lumen diameter and organ tone
Native structure	Multinucleated aligned fibers	Branched cardiomyocytes in laminar sheets	Spindle-shaped cells in aligned bundles or sheets
Tissue stiffness (kPa)	5–30 kPa (Gennisson et al., 2010; Feng et al., 2018)	5–20 kPa (Engler et al., 2008; Jacot et al., 2010)	1–30 kPa (Qiu et al., 2010; Petit et al., 2022)
Activation trigger	Motor neuron, action potentials	Sinoatrial node pacemaker activity with autonomic modulation	Nerves, hormones, stretch, local signals
Specific force (kN m^{-2})	100–700 (Krivickas et al., 2011; Roche et al., 2015)	30–60 (Tenreiro et al., 2021)	250–500 (Gabella, 1976)
Experiences fatigue	Yes	No	No
Regenerative capacity	High	Very low	Moderate

The high regenerative capacity of skeletal muscle is due to the presence of satellite stem cells that are activated upon injury, proliferate as myoblasts, and fuse to repair or replace damaged myofibers during myogenesis (Mukund and Subramaniam, 2020). In cases of volumetric muscle loss or muscle-wasting diseases, the endogenous regenerative capacity of skeletal muscle may become insufficient, making skeletal muscle tissue engineering a promising therapeutic strategy (Cordelle et al., 2025; Qi et al., 2025).

A first challenge in engineering skeletal muscle tissues is the reconstruction of the aligned structure of native muscle tissue (King et al., 2004). Anisotropic myoblast and myotube alignment is essential for achieving maximum force output and a coordinated contractile response, and can be promoted through biofabrication strategies, *in vitro* stimulation, or combined approaches (Fan et al., 2025).

A second challenge is achieving mature engineered skeletal muscle tissues. Mechanical, electrical, perfusion-based, or multimodal stimulation strategies have been shown to enhance myotube alignment, contractile force generation, and myogenic expression (Williamson et al., 2024; Cordelle et al., 2025; Fan et al., 2025). However, engineering skeletal muscle tissue still results in incomplete structural and functional maturation. Engineered constructs typically exhibit smaller myotube diameters, persistent expression of embryonic and prenatal myosin heavy chain isoforms, and lower tetanic-to-twitch ratios of about 2–4 compared to 3–16 in native skeletal muscle (King et al., 2004; Cheng et al., 2014). This is reflected in the force generation, with engineered skeletal muscle tissues typically producing specific tetanic forces of 0.05–50 kN m^{-2} compared to 100–700 kN m^{-2} in native skeletal muscle (Krivickas et al., 2011; Roche et al., 2015; Vesga-Castro et al., 2022).

Achieving physiological and translational relevance within skeletal muscle tissue engineering remains challenging. Immortalized

C2C12 murine myoblasts remain among the most widely used cell sources in engineered skeletal muscle models due to their robustness and ease of expansion (Zhao et al., 2023). However, reliance on murine cell lines may limit the ability of these models to accurately represent human muscle physiology and disease, highlighting the need for increased use of primary human cells and stem cell-derived muscle models.

These challenges highlight a functional gap between engineered and native skeletal muscle, indicating the need for improved integration of biofabrication strategies with physiologically relevant bioreactor stimulation to promote tissue maturation.

2.2 Cardiac muscle structure and function

The human heart contracts rhythmically enabling blood circulation, nutrient delivery, and removal of metabolic waste products throughout the body (Bornstein et al., 2024). Structurally, the heart consists of the contractile myocardium, which is flanked by an outer protective epicardium and inner endocardium (see Figure 3). Although cardiomyocytes occupy approximately 70%–85% of myocardial volume, they account for only a fraction of cardiac cells, with endothelial cells, fibroblasts, pericytes, and other supporting cell populations playing essential roles in myocardial function (Zhou and Pu, 2016; Litvinukova et al., 2020) Cardiomyocytes are branched, electrically excitable cells that connect through intercalated discs, enabling mechanical coupling, electrical impulse propagation and synchronized contraction (Nielsen et al., 2023). Cardiac contraction is initiated by the spontaneous electrical pulses generated in the sinoatrial node and propagated throughout the cardiac conduction system (Choudhury et al., 2015).

Myocardial fiber orientation varies across the ventricular wall and is commonly described as a transmural helical continuum, although alternative models of myocardial architecture have also been proposed (Grant, 1965; Dileep et al., 2023). The myocardium has high metabolic demands and relies on a dense coronary vascular network for oxygen and nutrient delivery (Riley and Smart, 2011; Bornstein et al., 2024). Moreover, the adult myocardium has limited regenerative capacity, with cardiomyocyte turnover remaining low throughout life (Bergmann et al., 2009; Bergmann et al., 2015). Consequently, the recreation of structurally and functionally mature myocardium remains a central objective in cardiac tissue engineering.

A first objective of engineered cardiac tissues is to recapitulate the anisotropic organization of the native myocardium. Cardiomyocyte alignment has been shown to promote sarcomere organization, electrical coupling, and force generation in engineered cardiac tissues (Takada et al., 2022; DePalma et al., 2024; Jain et al., 2024). Consequently, efforts have focused on the development of biofabrication strategies and bioreactor conditioning protocols, either independently or in combination, to direct cardiomyocyte alignment and tissue anisotropy (Bravo-Olin et al., 2024; Liu et al., 2026).

A second challenge is achieving mature engineered cardiac tissues. Mechanical, electrical, perfusion-based, or multimodal stimulation strategies have been shown to improve cardiomyocyte alignment, contractile force generation, synchronous contractions, and calcium handling (Bravo-Olin et al., 2024). Nevertheless, a functional gap remains between engineered and native myocardium. Engineered cardiac tissues typically generate twitch specific forces of approximately 0.1–3 kN m^{-2} compared with 30–60 kN m^{-2} of native adult myocardium (Tenreiro et al., 2021). Furthermore, conduction velocities generally remain around 0.1–0.15 m s^{-1} compared with 1–1.6 m s^{-1} in adult myocardium (Tenreiro et al., 2021).

Achieving physiological relevance represents a third challenge. The native myocardium is characterized by a heterogeneous multicellular environment and high metabolic demand. Therefore, engineered cardiac tissues require adequate oxygen and nutrient transport through appropriate construct design, vascularization, and perfusion strategies (Liu et al., 2026). In addition, engineered cardiac tissues require a biomimetic extracellular matrix with physiologically relevant mechanical properties, ideally reflecting the stiffness of native myocardium, which typically falls within the range of approximately 5–20 kPa, based on reported values of 6–10 kPa for embryonic and healthy myocardium and 18 ± 2 kPa for adult healthy myocardium (Jacot et al., 2010; Ribeiro et al., 2015).

Together, these challenges indicate the functional gap between engineered and native myocardium, emphasizing the need for integrated biofabrication and bioreactor-based conditioning strategies capable of recapitulating the structural, functional, and metabolic characteristics of native cardiac tissue.

2.3 Smooth muscle structure and function

Smooth muscle tissue is specialized for slow, sustained, and involuntary contractions (Figure 4), and is found throughout the gastrointestinal tract, vasculature, respiratory airways, urinary bladder, and reproductive system, where it regulates lumen diameter, peristalsis, and organ tone. Smooth muscle cells lack striations due to the absence of highly organized sarcomeres (Gartner and Hiatt, 2011). Instead, contractile proteins are anchored to α-actinin-containing dense bodies, which provide structural support and facilitate force transmission during contraction (Lehman and Morgan, 2012). Smooth muscle contractions are regulated by a combination of mechanical stimuli, autonomic innervation, hormonal signaling, and paracrine factors (Sanders, 2008; Brozovich et al., 2016). Consequently, smooth muscle tissues are exposed to tissue-specific biophysical and biochemical microenvironments, contributing to heterogeneity among vascular, gastrointestinal, urinary, airway, and reproductive smooth muscle tissues.

A key aspect of smooth muscle tissue is its phenotypic plasticity. Smooth muscle cells can reversibly transition between a synthetic phenotype, associated with proliferation, migration, and ECM remodeling, and a contractile phenotype specialized for force generation (Elmarasi et al., 2024). While this phenotypic switching contributes to tissue regeneration and repair, maintaining the contractile phenotype remains a major challenge in smooth muscle tissue engineering, as cells frequently adopt the synthetic phenotype during *in vitro* culture (Beamish et al., 2010). Therefore, biophysical cues, including mechanical conditioning, are often incorporated to promote contractile differentiation and functional maturation (Baldwin et al., 2024).

Reproducing the spatial organization of native smooth muscle tissue is another requirement for engineering smooth muscle tissues. Native smooth muscle tissue is typically organized into circumferential and longitudinal cell layers that coordinate contractions, such as vascular tone regulation and gastrointestinal peristalsis (Shea-Donohue et al., 2012; Elmarasi et al., 2024).

The diversity of smooth muscle tissues found in vascular, gastrointestinal, urinary, airway, and reproductive systems poses

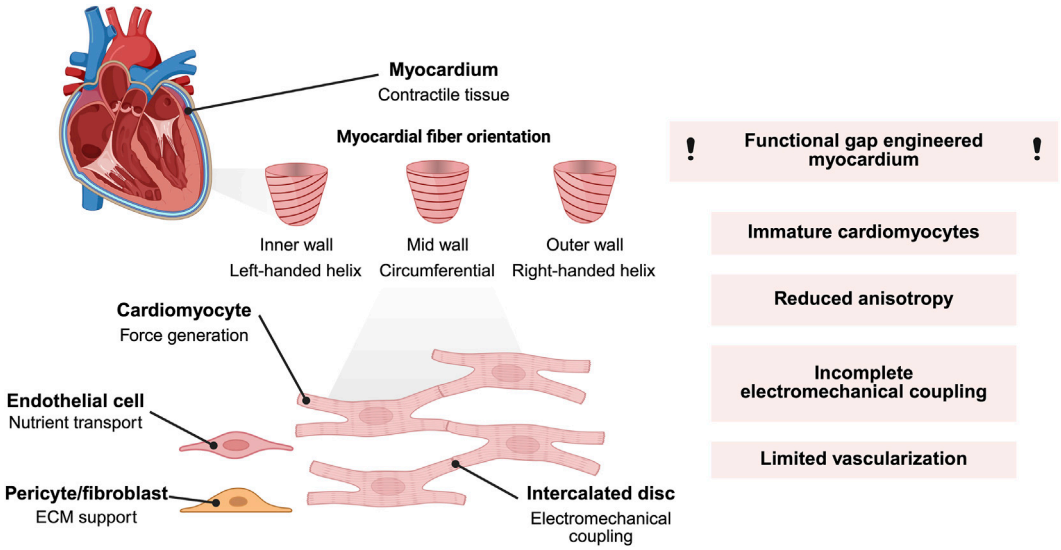

FIGURE 3
Native cardiac muscle structure and the functional gap observed in engineered cardiac tissues. Native myocardium is an anisotropic contractile tissue composed of cardiomyocytes, endothelial cells, fibroblasts, and pericytes, with coordinated force generation and electrical propagation enabled by intercalated discs and transmurally varying fiber orientation. Engineered cardiac tissues frequently exhibit immature cardiomyocytes, reduced anisotropy, incomplete electromechanical coupling, reduced force generation, and limited vascularization. Created in BioRender.

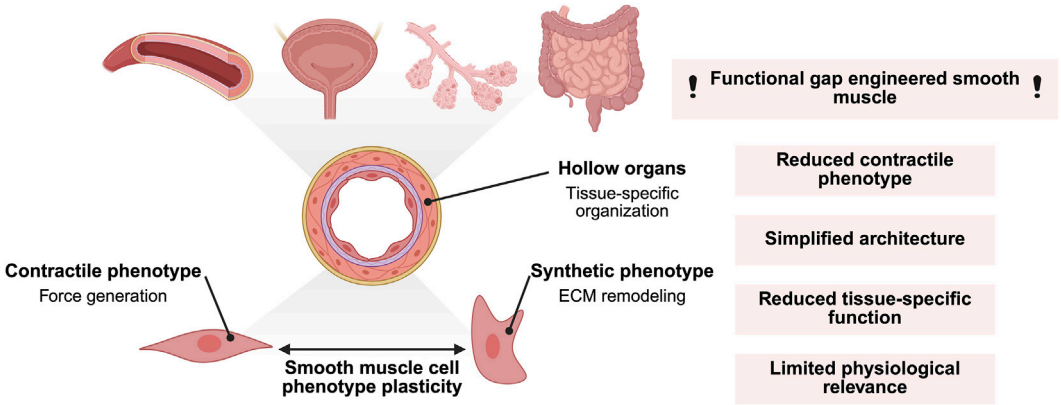

FIGURE 4
Native smooth muscle structure and the functional gap observed in engineered smooth muscle tissues. Smooth muscle exhibits tissue-specific organization within hollow organs and phenotype plasticity, allowing reversible transitions between contractile and synthetic phenotypes. While the contractile phenotype is specialized for force generation, the synthetic phenotype is associated with extracellular matrix remodeling. Engineered smooth muscle tissues frequently exhibit a reduced contractile phenotype, simplified architecture, reduced tissue-specific functionality, and limited physiological relevance compared with native tissue. Created with BioRender.

an additional tissue engineering challenge, as their distinct structural and functional requirements necessitate tissue-specific biofabrication and maturation strategies.

3 Biofabrication techniques for muscle tissue engineering

Tissue engineering and regenerative medicine have been employed to overcome current clinical challenges in muscle regeneration, while advancing relevant research fields such as disease modelling, biorobotics, and cultured meat (Albrecht et al., 2024; Kim et al., 2025; Liu et al., 2026). Engineering functional muscle tissue can be achieved using the tissue engineering triad consisting of cells, a biomaterial scaffold supporting 3D culture, and relevant signals including biophysical cues and chemical mediators (Groll et al., 2016). Of these three components, biomaterials have received significant research interest as they act as a supporting matrix for the developing tissue, actively instructing cell fate and matrix deposition (Levato et al., 2020).

Biofabrication encompasses the automated generation of biologically functional constructs using cells, biomaterials, and bioactive components through bioprinting, bioassembly, and subsequent maturation processes (Groll et al., 2016). Cell-laden biomaterials, termed bioinks, are central to this process as they need

to simultaneously meet biofabrication requirements while supporting cellular function and viability. Bioinks suitable for biofabrication possess physicochemical and biological properties that lie within the biofabrication window (Malda et al., 2013; Levato et al., 2020).

An essential requirement for muscle tissue engineering, is that the selected bioinks provide the necessary biochemical and biophysical cues to reproduce the native muscle microenvironment (Levato et al., 2020). The choice of the biofabrication technique suitable for each application depends on the muscle type, bioink properties, construct complexity and dimensions, fabrication resolution, and the desired mechanical properties (Samandari et al., 2022).

Because biofabrication determines construct architecture, mechanical properties, and cellular organization, it plays a critical role in directing tissue development and maturation (Cho et al., 2022; Baldwin et al., 2024; Fan et al., 2025). The following sections explore how extrusion and light-based biofabrication strategies are currently utilized to meet these complex demands across different muscle types (see Table 2).

3.1 Extrusion-based bioprinting

Extrusion bioprinting is an additive manufacturing technique that produces cell-laden scaffolds through layer-by-layer deposition of bioinks using pneumatic or mechanical extrusion. Owing to its compatibility with a broad range of biomaterials and cell densities, extrusion bioprinting is widely used to fabricate centimeter-scale tissue constructs (Ramesh et al., 2021; Rossi et al., 2024; Holland, 2025).

During extrusion, bioinks are exposed to shear stresses that influence cell viability, phenotype, and behavior while also promoting cellular and fibrillar alignment (Boularaoui et al., 2020). Such alignment is particularly important for the fabrication of anisotropic skeletal, cardiac, and smooth muscle tissue constructs, where cellular orientation is required to recapitulate native tissue architecture and function (Jain et al., 2024; Fan et al., 2025; Demri et al., 2026a). In addition, extrusion bioprinting enables the spatially controlled deposition of multiple biomaterials and cell types, facilitating the fabrication of complex multicellular tissue constructs (Cui et al., 2020). Commonly used biomaterials include gelatin, collagen, fibrin, decellularized ECM, and their derivatives, which support cellular differentiation and tissue maturation (Luo W. et al., 2024; Zhang et al., 2024; Holland, 2025). For complex geometries or low-viscosity bioinks, extrusion bioprinting can be combined with support-bath approaches such as FRESH to prevent structural collapse during fabrication (Hinton et al., 2015; Piñan Basualdo et al., 2024).

Despite its versatility, extrusion bioprinting remains limited by its relatively low resolution, typically about 100 μm, as well as the trade-off between print fidelity and cell viability, since higher printing pressures and smaller nozzle diameters can increase shear stresses, compromising cell survival and function (Samandari et al., 2022; Xu et al., 2022).

3.1.1 Extrusion bioprinting of skeletal muscle

Extrusion bioprinting is an established biofabrication strategy for skeletal muscle tissue engineering due to its versatility, scalability, and compatibility with a wide range of biomaterials and cell types (see Figure 5 and Table 2).

A key advantage of extrusion-based approaches is the generation of aligned cellular architectures through shear-induced orientation during printing, which promotes myoblast alignment, fusion, and myotube formation (Distler et al., 2020; Hwangbo et al., 2022). Additional structural guidance can be achieved through aligned fibrin microthreads and fibrous reinforcement materials, further enhancing anisotropic tissue organization and myogenic maturation (Lee et al., 2025; Heilig et al., 2026).

In addition to cellular alignment, extrusion bioprinting has enabled the fabrication of increasingly complex muscle constructs through multimaterial printing, sacrificial bioinks, coaxial core-shell architectures, and synthetic support frameworks (Kim et al., 2018; Filippi et al., 2023; Lee et al., 2023; Qiu et al., 2025). These strategies facilitate the generation of larger and more biomimetic tissues with improved nutrient transport, mechanical stability, and architectural organization. Several studies have further evaluated extrusion-bioprinted muscle constructs in volumetric muscle loss models, demonstrating varying degrees of vascularization, innervation, and functional recovery following implantation (Kim et al., 2018; Hwangbo et al., 2022; Fornetti et al., 2023; Lee et al., 2023).

Despite these advances, most extrusion-bioprinted skeletal muscle studies continue to focus primarily on cell viability, alignment, and myogenic differentiation rather than quantitative measures of tissue function. Although increasing reports of functional outcomes, including contractility and force recovery following implantation (Kim et al., 2018; Lee et al., 2023), the field remains dominated by morphological and molecular endpoints such as viability, alignment, fusion index, and myogenic marker expression. Furthermore, only a limited number of studies have combined extrusion bioprinting with bioreactor-based conditioning strategies, including perfusion systems and mechanically stimulated culture platforms (Filippi et al., 2023; Loi et al., 2024) Consequently, the interactions between construct architecture, biophysical stimulation, and long-term skeletal muscle maturation remain insufficiently understood and represent an important area for future research.

3.1.2 Extrusion bioprinting of cardiac muscle

A major challenge in cardiac extrusion bioprinting (see Figure 5 and Table 2) is the recreation of functional myocardial tissue, which requires high cardiomyocyte viability alongside electromechanical coupling, multicellular interactions, vascular support, and sustained synchronized contractile activity (Cahall et al., 2020; Akter et al., 2025). Consequently, cardiac bioinks must balance print fidelity, low shear stress, and high cell viability while providing biomechanical cues that support tissue maturation (Duan, 2017; Hospodiuk et al., 2017; Xuan et al., 2023). Moreover, extracellular matrix properties play a key role in cardiomyocyte organization, maturation, force transmission, and mechanosensing (DePalma et al., 2024).

A primary objective of cardiac extrusion bioprinting has been the fabrication of structurally complex myocardial tissues capable of supporting coordinated electromechanical activity (Esser et al., 2023; Mao et al., 2023). Using extrusion and support-bath approaches such as FRESH, increasingly complex ring-shaped, ventricle-like, and patient-specific cardiac constructs have been fabricated (Lee et al., 2019; Zhang et al., 2022; Esser et al., 2023; Mao et al., 2023; Hwang et al., 2024; Fang et al., 2026). These

TABLE 2 Overview of skeletal, cardiac, and smooth muscle extrusion and light-based biofabrication studies including the fabrication method, materials, cell types, key structural features of the scaffold, major findings, and the presence or absence of a bioreactor.

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Skeletal	Extrusion	Fibrin, gelatin, HA, PCL	Human primary muscle progenitor cells (hMPCs)	Multilayered aligned muscle bundles with sacrificial microchannels and PCL support anchors	Enhanced cell viability, myotube alignment, sarcomere organization, expression of mature myogenic markers, as well as innervation, vascularization, and volumetric and functional recovery following implantation in a volumetric muscle loss model	N.A.	(Kim et al., 2018)
Skeletal	Extrusion	GelMA	C2C12 murine myoblasts, human adipose-derived stem cells (hASCs)	Aligned cell-laden hydrogel struts via photocrosslinking during extrusion	Enhanced cell alignment, expression of mature myogenic markers as well as improved muscle regeneration and functional recovery upon implantation in a VML model	N.A.	(Hwangbo et al., 2022)
Skeletal	Extrusion	GelMA, alginate, Pluronic F127, PEGDA	C2C12 murine myoblasts	Perfusable microchannel network integrated with tendon-like anchors enabling passive tension during maturation	Perfusable constructs reduced hypoxia, improved cell viability, myotube alignment, fusion and expression of mature myogenic markers	N.A.	(Filippi et al., 2023)
Skeletal	Extrusion	GelMA, HAGM	C2C12 murine myoblasts, human tonsil-derived stem cells (hTMSCs)	Artificial muscle fascicles with core-shell structure	Enhanced cell–cell interaction and rapid myotube maturation, as well as functional recovery in a rat volumetric VML model upon implantation	N.A.	(Lee et al., 2023)
Skeletal	Extrusion	Fibrin, PEG	Human skeletal muscle-derived MSCs (hMSCs), murine mesoangioblasts	Aligned bundled microfibers deposited on custom C-shaped support mimicking native muscle fascicles	Enhanced myotube alignment, width, as well as vascularization, and innervation upon implantation in a VML model	N.A.	(Fornetti et al., 2023)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Skeletal	Extrusion	GelMA, AlgMA, fibrin	C2C12 murine myoblasts, human skeletal muscle myoblasts (HSMM)	Ring-shaped tissues	Supported high cell viability, alignment, multinucleated myotube formation, expression of mature myogenic markers, contractile activity, sarcomere formation and responsiveness to electrical stimulation	N.A.	(Garcia-Lizarribar et al., 2023)
Skeletal	Extrusion	Fibrin, alginate	C2C12 murine myoblasts	Extrusion bioprinted cell-laden hydrogel in elastic support for anchoring into uniaxial stretch bioreactor	High cell viability and increased mature myogenic marker expression	Cyclic strain	(Loi et al., 2024)
Skeletal	Extrusion	Fibrin	C2C12 murine myoblasts	Aligned fibrin threads, fabricated via extrusion in a coagulation bath, stretched and mounted on PDMS rings	Induced cellular alignment, maintained high viability, and promoted myotube formation and maturation	N.A.	(Lee et al., 2025)
Skeletal	Extrusion	Fibrin, gelatin, PCL	C2C12 murine myoblasts	PCL microfiber-reinforced porous construct with aligned fibrin filaments and tunable anisotropic mechanics through consecutive hybrid bioprinting	Microfiber reinforcement prevented construct shrinkage, promoted cell alignment, fusion, expression of mature myogenic markers, and generated native muscle-like tissue-level mechanical anisotropy	N.A.	(Qiu et al., 2025)
Skeletal	Extrusion	PEG, fibrinogen, alginate	Human skeletal muscle-derived pericytes	Aligned core-shell hydrogel fiber bundles via rotary wet-spinning	Enhanced alignment, myotube maturation, spontaneous contractility, ECM production, vascularization and muscle regeneration in VML model	N.A.	(Reggio et al., 2025)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Skeletal	Extrusion	GelMA, PCL	C2C12 murine myoblasts	Melt electrowritten fibrillar bundles incorporated into bioink and oriented during extrusion through shear flow creating an anisotropic microenvironment	Fibrillar bundles promoted cell attachment, myotube alignment, improving morphological maturation and anisotropy	N.A.	(Heilig et al., 2026)
Skeletal	FLight	GelNB, PEGSH, GelMA, HAMA, AlgMA	C2C12 murine myoblasts, NHDFs, tenocytes, HUVECs	Tubular scaffolds with aligned hydrogel microfilament networks and interconnected microchannels	Preserved cell viability, promoted cell alignment, nuclear elongation, collagen deposition, capillary alignment, and maturation	N.A.	(Liu et al., 2022)
Skeletal	FLight	Collagen, fibrinogen	Primary Pax7 murine myoblasts	Tubular scaffolds with aligned hydrogel microfilament networks and interconnected microchannels	Enhanced myotube alignment, maintenance of $Pax7^+$ stem cell pool, spontaneous and electrically induced contractions, and regenerative capacity after cardiotoxin injury	N.A.	(Liu et al., 2025)
Cardiac	Extrusion	Collagen	hESC-derived cardiomyocytes, cardiac fibroblasts	Freeform reversible embedding of suspended hydrogels (FRESH) printed cardiac constructs with perfusable vasculature and ventricular geometry	Synchronous contractions, directional electrical propagation, wall thickening during systole, perfusable vascular networks	Perfusion	(Lee et al., 2019)
Cardiac	Extrusion	HBC frame, fibronectin/gelatin nanofilms	hiPSC-derived cardiomyocytes, human cardiac fibroblasts, cardiac microvascular endothelial cells	Oriented rectangular cardiac tissue with aligned vascular network formed within printed HBC frame	Improved cell alignment, vascular network organization, synchronized contraction, and conduction velocity	N.A.	(Tsukamoto et al., 2020)
Cardiac	Extrusion	Porcine cardiac dECM, Laponite-XLG nanoclay, PEGDA	Human cardiac fibroblasts, hiPSC-derived cardiomyocytes	Extrusion-bioprinted cardiac dECM scaffold with tunable stiffness ranging from healthy-to-fibrotic myocardium and multilayer printability	High shape fidelity, cell viability, and tunable stiffness	N.A.	(Shin et al., 2021)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Cardiac	Extrusion	Spheroids supported within self-healing adamantane-cyclodextrin-based host-guest hydrogel	hMSCs, hiPSC-derived cardiomyocytes, cardiac fibroblasts	Spatially patterned spheroid-fusion cardiac microtissues with defined fibrotic regions	Reproduced cardiac fibrosis with reduced contractility, impaired electrical responsiveness, calcium handling, and controlled tissue heterogeneity	N.A.	(Daly et al., 2021)
Cardiac	Extrusion	PLLA vascular scaffold coated with Matrigel	hESC-derived cardiomyocytes, endothelial cells	Vascularized cardiac tissue printing on PLLA scaffold using an oil bath and a multi-axis robot	Maintained cell viability, supported vascularization, formed contractile cardiac tissue, and maintained long-term function over 6 months	Perfusion	(Zhang et al., 2022)
Cardiac	Extrusion	Collagen, HA	hiPSC-derived cardiomyocytes	Directly bioprinted ring-shaped and ventricle-shaped cardiac constructs in support bath	Long-term contractile cardiac tissues over 100 days, organized sarcomeres, synchronized contractions, contractile function, pharmacological responsiveness	N.A.	(Esser et al., 2023)
Cardiac	Extrusion	Fibrin	hiPSC-derived cardiomyocytes, hiPSC-derived endothelial cells, primary adult ventricular cardiac fibroblasts (hCFs)	High-density multicellular cardiac tissue ring printed around support posts in support bath	Cardiomyocyte alignment, calcium handling, conduction velocity, and pharmacological responsiveness	N.A.	(Finkel et al., 2023)
Cardiac	Extrusion	GelMA, AlgMA, reduced graphene oxide	Neonatal rat cardiomyocytes, cardiac fibroblasts, HL-1 cardiomyocytes	Simple ring-shaped conductive hydrogel cardiac construct; Ring-shaped cardiac BioRings printed in 12-well format; closed circular ends and filamentous ring strands intended to distribute force and promote alignment along print direction	Maintained cell viability, proliferation, alignment, gap junction formation, and calcium transients	N.A.	(Mousavi et al., 2024)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Cardiac	Extrusion	GelMA, PCL nanofibers	hiPSC-derived cardiomyocytes	3D aligned nanofiber scaffold enabling embedded extrusion bioprinting and anisotropic tissue organization	Improved cardiomyocyte alignment, elongation, and sarcomere organization, and gap junction formation	N.A.	(Wu et al., 2024)
Cardiac	Extrusion	GelMA, gelatin, fibrin, Pluronic	HUVECs	3D bioprinted perfusable vascular cardiac patch using sacrificial ink with endothelialized bifurcating channels	Supported the formation of a uniform endothelium. Reduction of scar formation, left ventricle dilation, and wall thinning, increased vascularization and proliferation of cardiomyocytes following implantation into a rat myocardial infarction model	Perfusion	(Hwang et al., 2024)
Cardiac	Extrusion	Collagen, Matrigel, alginate, fibrin	Neonatal rat ventricular cardiomyocytes, HUVECs	Helically aligned multilayered myocardium by combining microfluidic spinning with dual-axis fiber collection	Improved cardiomyocyte alignment, sarcomere organization, gap junction formation, synchronized contraction, endothelial network formation, and reduced fibrosis following implantation into a rat myocardial infarction model	N.A.	(Fang et al., 2026)
Cardiac	μCOP	GelMA, HAGM, PEGDA	Neonatal rat cardiomyocytes	μCOP-bioprinted anisotropic parallel microfibers integrated with cantilever force gauge	Anisotropic patterning improved cardiomyocyte alignment, sarcomere organization, synchronous beating, calcium handling, contractile force generation compared to non-aligned controls	N.A.	(Liu et al., 2020)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Cardiac	Volumetric	Porcine heart-derived dECM	Neonatal rat cardiomyocytes	Cardiomyocyte-laden heart geometry	High cell viability, spreading, sarcomere expression, and synchronized spontaneous contractions	N.A.	(Lian et al., 2024)
Cardiac	FLight	GelMA	Primary rat cardiomyocytes, hiPSCs	Filamented-light generated microfilaments producing controlled multidirectional cellular alignment and myocardium-inspired architectures	Improved cell alignment, gap junction formation, directed torsional contractions	N.A.	(Jones et al., 2024)
Cardiac	DLP	Cold-water fish GelMA functionalized with choline bio-ionic liquid	hiPSCs	Conductive hydrogel with triangle, square lattice, dermal papilla, ear and heart geometries	Sustained contractility up to 4 months, improved cardiac marker expression, sarcomere organization, calcium handling, gap junction formation, and spontaneous contractions	N.A.	(Banerjee et al., 2026)
Smooth	Extrusion	Fibrin, gelatin, HA	Rabbit bladder smooth muscle cells, bladder urothelial cells	Porous spiral tubular scaffold with cell-laden hydrogel layers	Native urethra-like mechanical properties, high cell viability, proliferation, maintenance of urothelial and smooth muscle biomarkers, and cell infiltration through scaffold pores	N.A.	(Zhang et al., 2017)
Smooth	Extrusion	Catechol-functionalized GelMA, Pluronic F127	Human coronary artery smooth muscle cells (HCASMCs), HUVECs, hMSCs	Self-standing, small-diameter vasculature with smooth muscle and endothelial lumen	Maintained high viability, supported endothelialization and smooth muscle differentiation, enhanced vasoreactivity, ECM deposition, perfusability, vascular remodeling, and *in vivo* host integration	N.A.	(Cui et al., 2019)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Smooth	Extrusion	Collagen, alginate, dECM	Primary human intestinal smooth muscle cells, primary human airway smooth muscle cells	Microfluidic 3D-bioprinted ring-shaped constructs	High viability, physiological contraction and relaxation responses, and disease-relevant remodeling response	N.A.	(Dickman et al., 2020)
Smooth	Extrusion	GelMA, HA	HUVECs, SMCs	Two-step crosslinked bilayer tubular scaffold consisting of an outer bioprinted SMC-laden GelMA vessel and endothelialized lumen	Supported high cell viability, formation of endothelial monolayer and smooth muscle layer, enabled fabrication of small-diameter vascular grafts with complex tubular geometries	N.A.	(Jin et al., 2022)
Smooth	Extrusion	Alginate, PEO	Human tonsil-derived MSCs	Aligned extrusion or cell-electrospun nanofibrous patch mimicking esophagus smooth muscle architecture	Cell electrospinning improved cell alignment, elongation, marker expression of the contractile SMC phenotype, as well as enhanced vascularization and wound regeneration upon implantation	N.A.	(Yeo et al., 2023)
Smooth	Extrusion	GelMA, HAMA, PEGDA	Rabbit adipose-derived stem cells (rADSCs)	Porous photocrosslinked extrusion-bioprinted scaffold	Supported high viability and proliferation, promoted smooth muscle differentiation by increased α-SMA and SM-MHC expression	N.A.	(Atturu et al., 2024)
Smooth	Extrusion	GelMA, silk-fibroin-MA, Fe_3O_4 nanoparticles	Primary rabbit smooth muscle cells (SMCs), bone marrow mesenchymal stem cells (BMSCs)	Multi-material, magnetically responsive, hierarchically structured anisotropic scaffold with engineered microarchitecture and functional alignment cues	Directed 3D cell alignment, high cell viability, promoted BMSC differentiation into SMCs, enhanced smooth muscle self-organization, improved intercellular connectivity and ECM deposition, accelerated in smooth muscle regeneration upon implantation	N.A.	(Luo et al., 2024b)

(*Continued*)

TABLE 2 Continued

Tissue	Fabrication method	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Bioreactor	References
Smooth	DLP	PEGDA-AAm	Smooth muscle cells (SMCs)	DLP-printed scaffolds with spiral grooves	Guided SMC alignment and organization through engineered surface topography and mechanical cues	N.A.	(Yang et al., 2024b)
Smooth	Volumetric	GelM, dECM, plasmonic resin	Human pulmonary artery smooth muscle cells (SMCs)	Sequential multimaterial volumetric bioprinting of multilayer vascular-inspired constructs with perfusable channels	Supported cell viability, proliferation, elongation, network formation	N.A.	(Aizarna-Lopetegui et al., 2025)

Abbreviations: AAm, acrylamide; AlgMA, alginate methacrylate; BMSCs, bone marrow mesenchymal stem cells; dECM, decellularized extracellular matrix; DLP, digital light processing; Fe_3O_4, ferric oxide nanoparticles; FLight, filamented light bioprinting; FRESH, freeform reversible embedding of suspended hydrogels; GelM, gelatin methacrylate; GelMA, gelatin methacryloyl; GelNB, gelatin norbornene; HA, hyaluronic acid; HAGM, hyaluronic acid glycidyl methacrylate; HAMA, hyaluronic acid methacrylate; HBC, hydroxybutyl chitosan; hASCs, human adipose-derived stem cells; HCASMCs, human coronary artery smooth muscle cells; hCFs, human cardiac fibroblasts; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; hMPCs, human primary muscle progenitor cells; hMSCs, human mesenchymal stem cells; HSMM, human skeletal muscle myoblasts; hTMSCs, human tonsil-derived stem cells; HUVECs, human umbilical vein endothelial cells; NHDFs, normal human dermal fibroblasts; PCL, poly (ε-caprolactone); PDMS, polydimethylsiloxane; PEG, poly (ethylene glycol); PEGDA, poly (ethylene glycol) diacrylate; PEGSH, poly (ethylene glycol) thiol; PEO, polyethylene oxide; PLLA, poly (L-lactic acid); rADSCs, rabbit adipose-derived stem cells; SMCs, smooth muscle cells; SM-MHC, smooth muscle myosin heavy chain; VML, volumetric muscle loss; α-SMA, alpha-smooth muscle actin; μCOP, micro-continuous optical printing.

engineered tissues exhibited organized sarcomere formation, spontaneous synchronized contractions, calcium transients, directional electrical propagation, and anisotropic cardiomyocyte alignment through the incorporation of topographical guidance cues and aligned fiber architectures (Lee et al., 2019; Zhang et al., 2022; Esser et al., 2023; Wu et al., 2024; Fang et al., 2026). Furthermore, anisotropic cardiomyocyte organization has been shown to increase tissue-level contractions, highlighting the functional importance of cardiomyocyte alignment (Tsukamoto et al., 2020).

To enhance cell-cell coupling and tissue function, multicellular bioinks containing cardiomyocytes, endothelial cells, fibroblasts, and other supporting cells, as well as spheroid- and aggregate-based strategies, have been used (Daly et al., 2021; Finkel et al., 2023). These approaches promoted tissue fusion, synchronized contraction, calcium-wave propagation, and physiologically relevant electromechanical behavior, while studies integrating biofabrication with dynamic maturation platforms further demonstrated enhanced tissue organization and functional performance (Esser et al., 2023; Finkel et al., 2023; Mousavi et al., 2024).

Overall, extrusion bioprinting has enabled the fabrication of increasingly functional cardiac tissues. However, limitations in vascularization, cellular maturation, scalability, and reproducibility continue to hinder clinical translation (Walcott and Davis, 2025). Furthermore, functional performance is often evaluated using electrophysiological metrics such as calcium handling and conduction velocity, whereas quantitative measurements of force generation remain scarce. Bioreactor systems may help address these challenges by providing controlled culture conditions that promote tissue maturation and long-term functionality.

3.1.3 Extrusion bioprinting of smooth muscle

Extrusion-based bioprinting has emerged as a versatile platform for smooth muscle tissue engineering (see Figure 5 and Table 2) owing to its ability to fabricate cell-laden constructs with controlled geometry, high cell density, and spatially organized architectures (Davoodi et al., 2020). Because smooth muscle function depends on tissue organization and phenotypic state, extrusion bioprinting strategies and bioink formulations must provide structural, mechanical, and biochemical cues that support smooth muscle maturation and maintenance of the contractile phenotype (Xuan et al., 2023).

A primary objective of extrusion bioprinting of smooth muscle has been the recreation of the native tissue architecture through the fabrication of multilayered, perfusable, and endothelialized constructs. Both extrusion and coaxial biofabrication strategies have enabled the generation of biomimetic vascular and hollow organ geometries featuring endothelialized lumens and organized smooth muscle layers (Cui et al., 2019; Jin et al., 2022; Jin et al., 2023). Structural guidance cues, including bioprinted microchannels, aligned microfibers, and magnetic-field-assisted organization, promoted anisotropic smooth muscle cell alignment, enhanced contractile smooth muscle phenotypes, and improved smooth muscle tissue organization (Tijore et al., 2018; Yeo et al., 2023; Luo Y. et al., 2024).

A second objective has been the development of physiologically functional smooth muscle tissues for regenerative medicine and disease modeling. Several extrusion-bioprinted constructs have been investigated as implantable tissues for urethral, vascular, and esophageal regeneration, where they promoted tissue integration, vascularization, and smooth muscle tissue formation *in vivo* (Cui

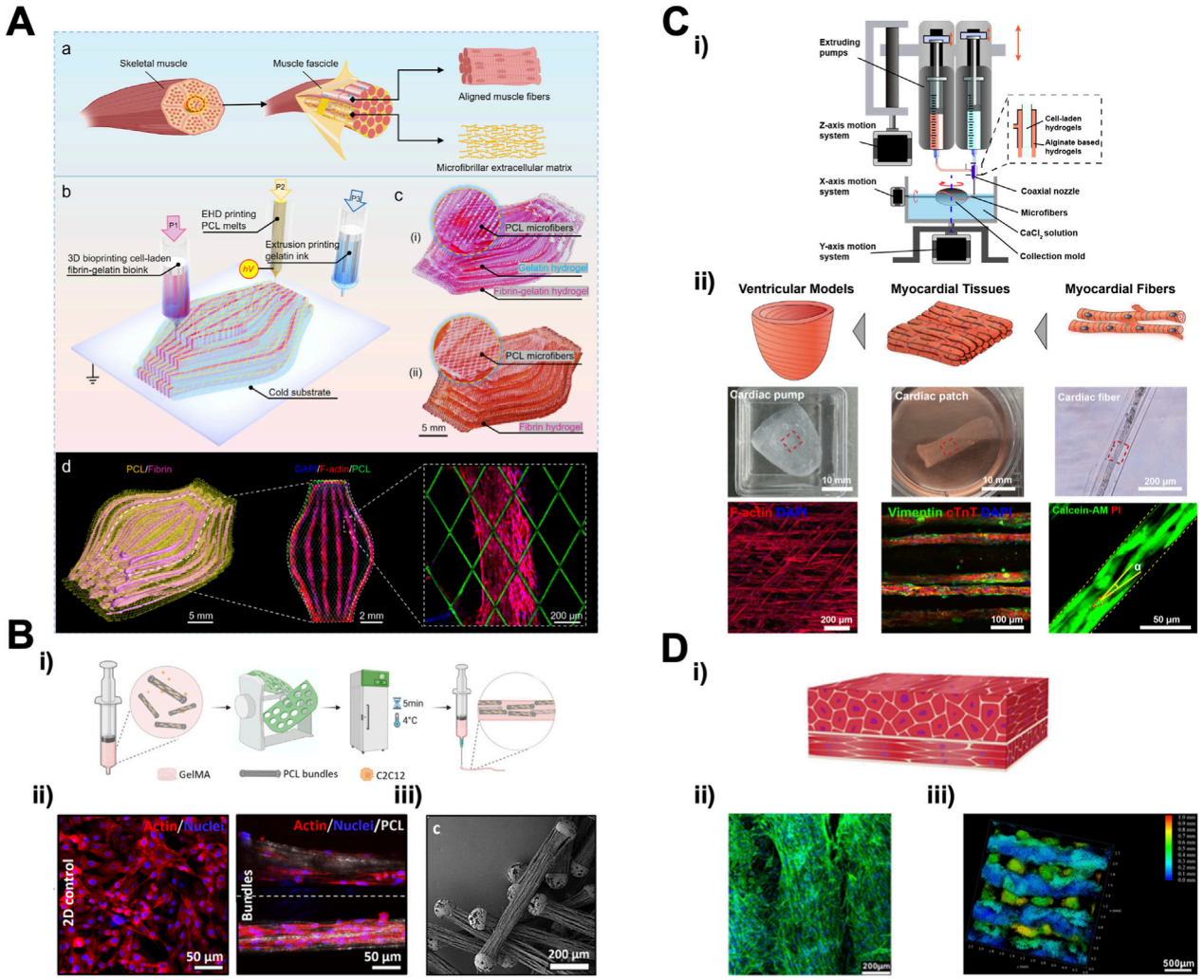

FIGURE 5
Overview of extrusion-based bioprinting strategies for engineering anisotropic muscle tissues. **(A)** Consecutive hybrid bioprinting (CHB) integrating cell-laden hydrogel filaments, sacrificial gelatin filaments, and structural PCL microfibers to generate anisotropic reinforced skeletal muscle constructs. Adapted from (Qiu et al., 2025), with permission. **(B)** Composite GelMA/PCL bioink approach enabling guided myoblast alignment. (i) Bioink preparation workflow, (ii) cytoskeletal alignment of C2C12 myoblasts within fibrillar bundles, (iii) SEM image of a laser-cut fibrillar bundle. Adapted from (Heilig et al., 2026), CC BY 4.0. **(C)** Flow-led assembly for spiral hierarchical structures (FLASH) enabling fabrication of aligned myocardial fibers, patches, and ventricular constructs through coaxial extrusion and directional collection in a rotating calcium chloride bath. Adapted from Fang et al. (2026), CC BY 4.0. **(D)** Magnetically assisted extrusion bioprinting for smooth muscle organization. (i) Schematic of the aligned scaffold design; (ii) cellular organization within Fe_3O_4 nanoparticle-containing hydrogels; (iii) reconstructed scaffold architecture. Adapted from (Luo Y. et al., 2024), CC BY 4.0.

et al., 2019; Yeo et al., 2023; Luo Y. et al., 2024). In parallel, bioprinted smooth muscle models exhibiting physiologically relevant and drug-responsive contractility have emerged as promising platforms for disease modeling and therapeutic screening, enabling investigation of both acute contractile responses and pathological tissue remodeling *in vitro* (Dickman et al., 2020).

While most studies report biological outcomes including cell viability, tissue organization, and phenotypic marker expression, quantitative assessment of contractile force generation remains scarce. Furthermore, studies combining extrusion bioprinting with mechanical, electrical, perfusion-based, or multimodal bioreactor conditioning remain scarce. Consequently, the relationships between construct design, contractile phenotype maintenance, and functional maturation remain poorly understood and require further investigation.

3.2 Light-based biofabrication

Light-based biofabrication is increasingly used to generate cell-laden tissues due to its high spatial resolution (see Figure 6), low shear stress, and compatibility with complex multimaterial or multicellular constructs (Prados-Martin et al., 2026). These characteristics are particularly relevant for muscle tissue engineering, where anisotropic architectures and precise cellular organization are required to recapitulate native tissue structure and function (Jain et al., 2024; Fan et al., 2025; Demri et al., 2026a). The main principle of light-based biofabrication is the spatially controlled photopolymerization of photosensitive bioinks, with different approaches offering distinct trade-offs between resolution and fabrication speed (Prados-Martin et al., 2026).

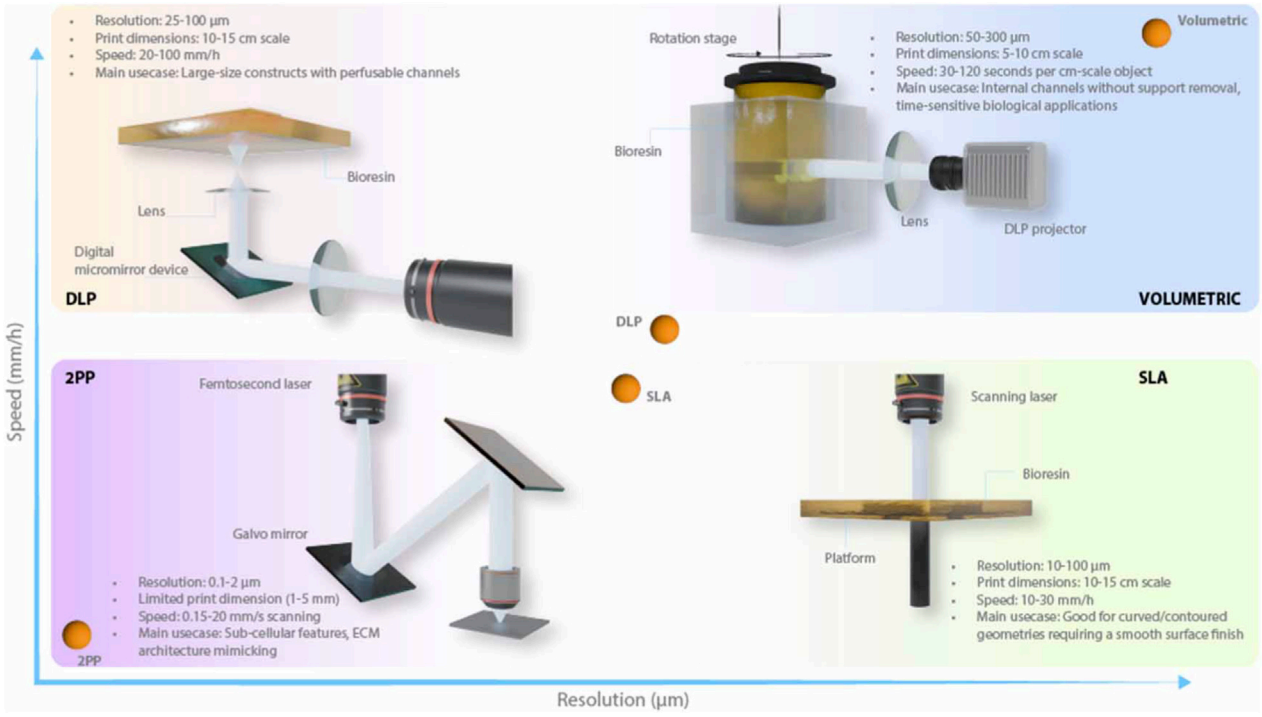

FIGURE 6
Overview of light-based biofabrication methods and their corresponding resolution and print speed. Abbreviations: digital light printing (DLP), two-photon polymerization (2 PP), stereolithography (SLA). Adapted from (Prados-Martin et al., 2026), CC BY 4.0.

Photosensitive bioinks typically consist of a hydrogel precursor, cells, and a photoinitiator, requiring a balance between printability and cell viability (Lim et al., 2020). Commonly used biocompatible wavelengths include 405 nm and emerging visible light-activated systems (Müller et al., 2022; Elkhoury et al., 2023).

Among light-based biofabrication techniques, stereolithography (SLA) and digital light projection (DLP) follow a point-by-point and layer-by-layer photopolymerization strategy, whereas two-photon polymerization (2 PP) enables sub-micron resolution (Song et al., 2020; Greant et al., 2023). In contrast, volumetric bioprinting prioritizes fabrication speed, enabling centimeter-scale constructs to be generated within seconds to minutes while maintaining geometric complexity (Jing et al., 2023; Bernal et al., 2025).

However, further advances in bioinks, multimaterial processing, and multicellular tissue biofabrication are required to fully exploit the potential of light-based biofabrication for muscle tissue engineering (Bernal et al., 2025; Prados-Martin et al., 2026).

3.2.1 Light-based biofabrication of skeletal muscle

One of the main applications of light-based biofabrication in skeletal muscle tissue engineering has been the recreation of aligned microarchitectures that mimic the anisotropic organization of native muscle tissue. Filamented light (FLight) bioprinting enables the rapid fabrication of hydrogel constructs containing highly aligned microfilaments and interconnected microchannels that guide cellular organization and support the formation of aligned, multinucleated, and contractile myotubes. Using this approach, engineered mini-muscles were generated that exhibited electrically induced twitch and tetanus-like contractions, assessed through contractile displacement measurements, and contained a resident Pax7^{+} progenitor population with regenerative potential following injury (Liu et al., 2022; Liu et al., 2025).

Despite these promising results, light-based biofabrication remains relatively underexplored in skeletal muscle tissue engineering, with only a limited number of studies reported to date (see Figure 7 and Table 2). Studies relying solely on photocrosslinking of extruded or cast bioinks were excluded from this review, as light does not directly govern construct architecture in such approaches. Furthermore, the application of bioreactor-based conditioning strategies to light-biofabricated skeletal muscle tissues may further enhance tissue maturation and functional development.

3.2.2 Light-based biofabrication of cardiac muscle

Light-based biofabrication has emerged as a versatile platform for cardiac tissue engineering by combining high spatial resolution, geometric freedom, and rapid hydrogel crosslinking (see Figure 7 and Table 2). In contrast to extrusion-based methods, these approaches largely avoid shear-induced stresses during construct fabrication, which can contribute to improved preservation of cell viability and function (Xu et al., 2022; Prados-Martin et al., 2026).

A primary objective of light-based biofabrication of cardiac muscle has been the generation of anisotropic microarchitectures that promote cellular organization and electromechanical coupling. Studies using DLP and microscale continuous optical printing (µCOP) approaches generated aligned cardiac tissues with

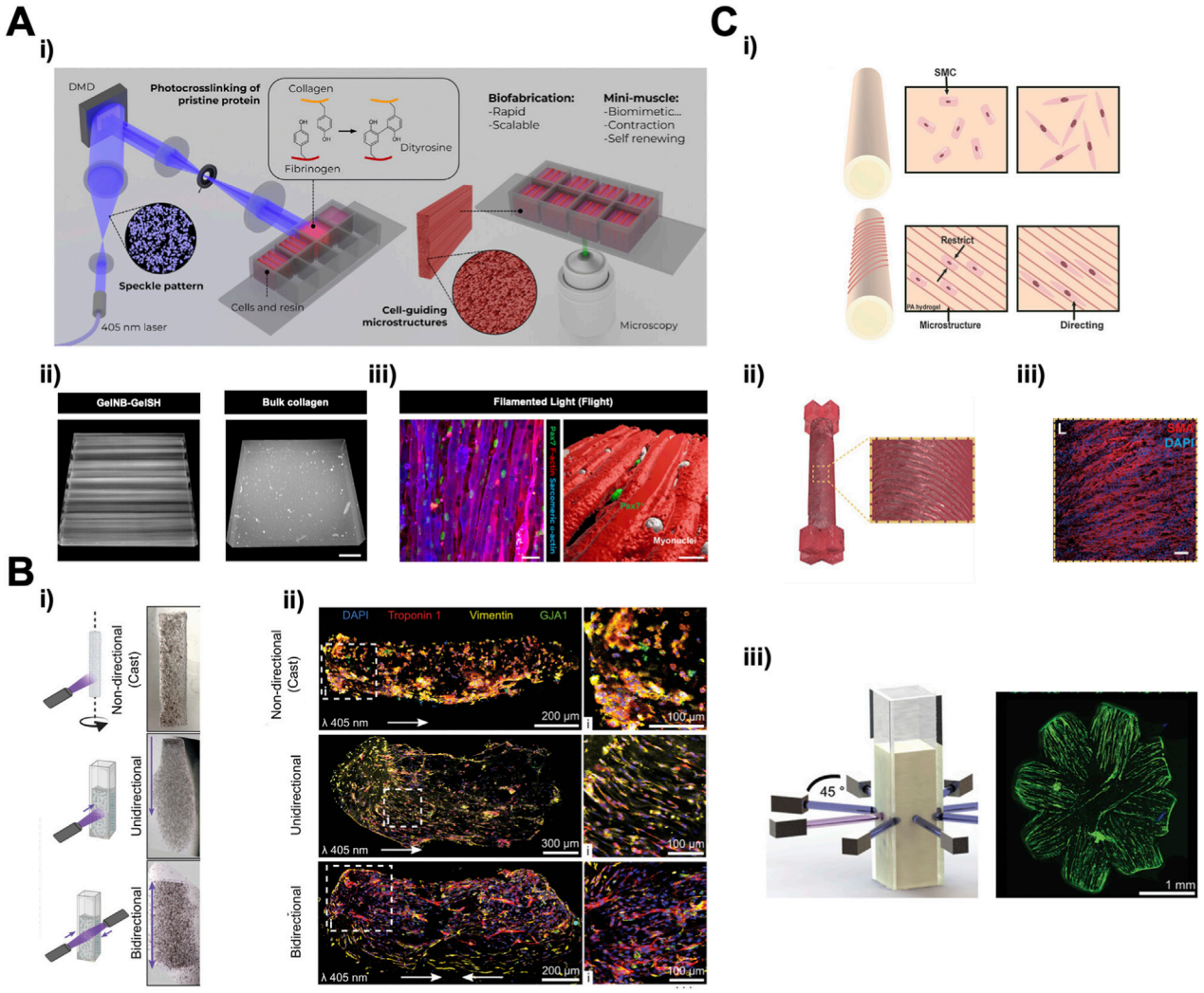

FIGURE 7
Overview of light-based bioprinting for muscle tissue engineering. **(A)** FLight printing of engineered mini-muscles using pristine protein-based photoresins. (i) Schematic illustration of the FLight fabrication process. (ii) Confocal images demonstrating matrix-dependent microarchitecture formation. (iii) Representative fluorescence and 3D reconstruction images showing aligned myotube formation and satellite cell maintenance in FLight-engineered muscle constructs. Adapted from (Liu et al., 2025), CC BY 4.0. **(B)** Light-guided control of cellular alignment and tissue architecture. (i) Schematic overview of non-directional, unidirectional, and bidirectional irradiation strategies. (ii) Immunostained cardiac tissues demonstrating enhanced cellular alignment following directional light exposure. (iii) Multidirectional illumination enables the fabrication of complex anisotropic tissue architectures, including multilayered myocardium and jellyfish-inspired constructs. Adapted from (Jones et al., 2024), CC BY 4.0. **(C)** Light-induced microtopographical guidance of muscle cell alignment. (i) Schematic illustration of cell orientation induced by microstructured PEGDA-AAm hydrogels. (ii) 3D-printed hydrogel tube containing periodic surface microfeatures for directional guidance. (iii) Fluorescence image showing smooth muscle alignment along the engineered surface topography. Adapted from (Yang M. et al., 2024), with permission.

organized sarcomeres, synchronous contractions, improved contractile force generation, and directional electrical propagation (Liu et al., 2020; Wang et al., 2021; Banerjee et al., 2026). These findings highlight the importance of architectural guidance cues for promoting cardiomyocyte alignment, electromechanical coupling, and tissue maturation.

A second focus has been increasing biological and structural complexity. Light-based biofabrication has enabled the printing of stem-cell-laden constructs that undergo cardiac differentiation following fabrication, as well as engineered heart tissues with tunable geometry, stiffness, and maturation states (Banerjee et al., 2026). More recently, volumetric bioprinting has emerged as a powerful strategy for the rapid fabrication of anatomically relevant cardiac tissues, enabling the use of biologically complex and tissue-specific bioinks while reducing manufacturing times from minutes to seconds. Resulting constructs demonstrated increased cardiomyocyte viability, spreading, sarcomere organization, synchronized contractions, calcium-wave propagation, and disease-modelling capabilities (Lian et al., 2024; Janssen et al., 2026). Moreover, FLight bioprinting has expanded the ability to generate biomimetic anisotropic tissue architectures, providing precise control over filament organization and cellular alignment while retaining the advantages of light-based manufacturing (Jones et al., 2024). Together, these approaches highlight complementary routes toward increasing structural complexity, biological functionality, and physiological relevance in engineered cardiac tissues.

Overall, light-based biofabrication has enabled the generation of functional cardiac tissue models with high geometric control and cellular organization. However, quantitative assessments of force generation, conduction velocity, vascularization, and long-term maturation remain comparatively limited. Advancing these functional outcomes will require platforms that integrate tissue fabrication with stimulation, perfusion, and real-time functional sensing.

3.2.3 Light-based biofabrication of smooth muscle

Light-based biofabrication strategies have primarily focused on recreating smooth muscle microenvironments through precise control over tissue architecture, mechanical properties, and cell organization (see Figure 7 and Table 2).

A primary objective of light-based biofabrication has been the recreation of native vascular heterogeneity through precise control of tissue architecture, cellular organization, and material composition. Multimaterial DLP and sequential volumetric bioprinting have enabled the fabrication of concentric vessel-like structures that mimic native vascular layers while maintaining high cell viability and spatial control over cell distribution (Yang M. et al., 2024; Aizarna-Lopetegui et al., 2025). Additionally, engineered topographical features, such as spiral grooves, have been used to direct smooth muscle cell alignment and promote anisotropic tissue organization (Yang M. et al., 2024).

A second objective has been the fabrication of physiologically relevant smooth muscle architectures. Volumetric bioprinting and DLP-based approaches have enabled the generation of perfusable vascular constructs and patient-specific tissue geometries with high cell viability, homogeneous cell distribution, and biomimetic structural organization (Yang M. et al., 2024; Aizarna-Lopetegui et al., 2025; Catano et al., 2026).

Collectively, these studies highlight the potential of light-based biofabrication to generate structurally complex and physiologically relevant smooth muscle tissues. Nevertheless, the induction and long-term maintenance of a mature contractile smooth muscle phenotype remain largely unexplored, representing a key challenge for future smooth muscle tissue engineering strategies combining biofabrication with bioreactor-based stimulation.

4 Bioreactor-based *in vitro* stimulation

Biofabrication strategies, such as extrusion and light-based bioprinting, primarily focus on recreating the structural and physicochemical properties of engineered tissues through scaffold architecture, material selection, cellular composition, and spatial patterning (Ramesh et al., 2021; Prados-Martin et al., 2026). These approaches can generate aligned cellular organizations, anisotropic tissue architectures, and increasingly complex multicellular constructs (Cui et al., 2020; Fan et al., 2025; Demri et al., 2026a). However, engineered muscle tissues often remain structurally and functionally immature, as current biofabrication approaches alone do not fully reproduce the dynamic biophysical cues required for muscle development and function (Fan et al., 2025; Demri et al., 2026a).

In contrast, bioreactor systems provide controlled environmental cues that drive tissue maturation and bridge the functional gap between engineered and native muscle (Bravo-Olin et al., 2024; Williamson et al., 2024). Mechanical, electrical, and perfusion-based stimulation can recapitulate key aspects of the dynamic *in vivo* microenvironment, influencing cellular organization, differentiation, and tissue function (Bravo-Olin et al., 2024; Williamson et al., 2024). Consequently, the integration of biofabrication with bioreactor-based conditioning has emerged as a promising strategy for developing more physiologically relevant muscle models. The following sections examine how specific mechanical, electrical, perfusion, and multimodal bioreactor systems are currently utilized to drive this functional maturation across different muscle types (see Table 3).

4.1 Mechanical stimulation

Mechanical cues play an important role in regulating muscle development, organization, and function (Vining and Mooney, 2017). Mechanical stimulation aims to partially recapitulate the dynamic loading conditions experienced by native muscle tissues, thereby promoting tissue maturation and reducing the functional gap between engineered and native muscle. Through mechanotransduction, externally applied forces regulate cellular organization, differentiation, extracellular matrix remodeling, and functional maturation across skeletal, cardiac, and smooth muscle tissues (Engler et al., 2006; Vining and Mooney, 2017).

To reproduce these stimuli *in vitro*, a variety of mechanical bioreactors has been developed, with strain-based bioreactors representing one of the most widely used approaches in muscle tissue engineering. These platforms apply controlled static or cyclic strain in uniaxial, biaxial, or equibiaxial configurations, with cellular responses highly dependent on factors including strain magnitude, frequency, and duration. Both static and cyclic loading have been widely used to promote muscle maturation, although their biological effects depend strongly on the applied stimulation parameters and engineered tissue environment (Bravo-Olin et al., 2024; Williamson et al., 2024).

4.1.1 Mechanical stimulation of skeletal muscle

When considering strain-based bioreactors, uniaxial stretching is the most widely applied mechanical stimulation modality for skeletal muscle tissue engineering (see Figure 8 and Table 3), as it reproduces the predominant mechanical loading direction experienced by skeletal muscle *in vivo* (Sunadome et al., 2023).

A first common stimulation strategy is static uniaxial strain, which consists of sustained static uniaxial tension, generated by culturing engineered muscle constructs between anchor posts, allowing cell-mediated matrix compaction to generate continuous mechanical tension. Such systems promote myotube alignment, tissue anisotropy, and expression of myogenic markers while also enabling quantitative assessment of contractile performance through cantilever deflection, post deflection, or force transducer-based measurements upon electrical stimulation (Madden et al., 2015; Vesga-Castro et al., 2022; Takahashi et al., 2023; Yoshida et al., 2025; Demri et al., 2026b). These findings are consistent with the role of continuous tensile loading during *in vivo* muscle formation where it regulates myotube polarization, elongation, and muscle directionality (Sunadome et al., 2023). As a result, static tension-

TABLE 3 Overview of skeletal muscle, cardiac muscle, and smooth muscle mechanical, electrical, perfusion, and multimodal bioreactor studies including the bioreactor type, materials, cell types, key structural features of the bioreactor, major findings, and the presence or absence of biofabrication.

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Static strain	Fibrin	Primary human skeletal muscle progenitor cells	Scaffold anchored within PDMS frames	Generated aligned myotubes, twitch and tetanic contractions, satellite stem cell population, calcium handling, functional acetylcholine receptors, and physiological response in response to small molecules	N.A.	(Madden et al., 2015)
Skeletal	Static strain	Fibrin	C2C12 mouse myoblasts	Bioreactor applying programmable static strain through magnetic force transmission	Promoted cell alignment, fusion index, sarcomere organization, myogenic differentiation, and hypertrophy	N.A.	(Heher et al., 2015)
Skeletal	Static strain	Collagen	Primary human myoblasts	96-well micro-muscle PDMS platform with two flexible pillars	Produced aligned myotubes, organized sarcomeres, contractile force, mature myogenic marker expression, optogenetic stimulation induced hypertrophy and metabolic maturation resembling exercise adaptation	N.A.	(Mills et al., 2019)
Skeletal	Static strain	Collagen	Primary rat skeletal myoblasts	Ring-shaped skeletal muscle scaffolds cultured under static stretch between metal hooks	Generated aligned and mature contractile muscle with a functional satellite cell niche and regenerative capacity upon implantation	N.A.	(Tiburcy et al., 2019)
Skeletal	Static strain	Fibrin	Primary human myoblasts	96-well culture PDMS platform containing two flexible vertical microposts	Generated aligned and mature myotubes with increasing contractile force and calcium handling over time, enabled drug screening and detected small molecule toxicity based on force generation	N.A.	(Afshar et al., 2020)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Static strain	Fibrin	Human skeletal muscle satellite cells	Thermoresponsive micropatterned PNIPAM substrate for aligned multilayer cell sheet fabrication inside support frame	Improved cell alignment, sarcomere organization, force generation, and physiological response for quantitative drug testing	N.A.	(Takahashi et al., 2023)
Skeletal	Static strain	Fibrin	hiPSC, human primary myoblasts	Custom PDMS culture chambers with two flexible pillars allowing for scaffold casting and force measurements	The engineered hiPSC skeletal muscle supported myofiber maturation and organization, and contractile forces comparable to primary myoblast-derived constructs	N.A.	(van der Wal et al., 2023)
Skeletal	Static strain	Matrigel	Healthy and Congenital Muscular Dystrophy primary human immortalized myoblasts	Micropillar-based muscle-on-chip with micropatterned guidance cues	Enabled spontaneous contraction monitoring and modeling of muscular dystrophy with reduced contractility	N.A.	(Rose et al., 2023)
Skeletal	Static strain	Fibrin	Healthy and DMD immortalized human muscle precursor cells	PDMS casting mold with a rectangular-shaped pool and two flexible pillars	Reproduced sustained contraction-induced sarcolemmal damage mirroring Duchenne muscular dystrophy (DMD) tissues, induced tetanic fatigue-like phenotype, enabled evaluation of utrophin-upregulating compounds	N.A.	(Tejedera-Villafranca et al., 2023)
Skeletal	Static strain	Fibrin	Primary human skeletal muscle cells (HSkMC)	Anchored muscle tissue with embedded pillar electrodes enabling localized electrical stimulation	Improved contractile distance and stimulation selectivity versus conventional electrodes, supported aligned myotube formation and contractile function measurement	N.A.	(Li et al., 2024)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Static strain	Fibrin	FSHD-hiPSCs	24 well-plate setup with T-bone-shaped PDMS chambers and pillars	Modelled facioscapulohumeral muscular dystrophy (FSHD) pathology with reduced muscle force and sarcomere organization in the presence of small molecules	N.A.	(Franken et al., 2025)
Skeletal	Static strain	Fibrin	Human skeletal muscle satellite cells (HSkMC)	Cell-laden sheet in support frame compatible with a load cell setup	Generated aligned myofibers, organized sarcomeres, contractile force generation, quantitative drug-response, and fatigue assessment	N.A.	(Yoshida et al., 2025)
Skeletal	Static strain	Collagen, maghemite iron oxide nanoparticles	C2C12 mouse myoblasts, hiPSC	Magnetic bioprinting and clipping system on magnetic needles within a chip for controlled stretching	Improved anisotropic tissue architecture, enhanced alignment, myoblast fusion, and maturation, sarcomere organization, calcium responsiveness, contractility, stretching accelerated myogenic differentiation and contractility	N.A.	(Demri et al., 2026b)
Skeletal	Cyclic strain	Collagen	C2C12 murine myoblasts	Mechanical stimulation bioreactor using a ramp and hold phase	Mechanical loading promoted myotube hypertrophy, fusion index, and increased contractile force generation	N.A.	(Aguilar-Agon et al., 2019)
Skeletal	Cyclic strain	Fibrin	C2C12 murine myoblasts	Bioreactor system using magnetic force transmission to apply uniaxial tensile strain to ring-shaped scaffolds	Cyclic tensile loading promoted myoblast alignment, activation, hypertrophy, fusion index, and mature myogenic marker expression	N.A.	(Tomasch et al., 2023)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Cyclic strain	Fibrin, alginate	C2C12 murine myoblasts	Extrusion bioprinted cell-laden hydrogel in elastic support for anchoring into uniaxial stretch bioreactor	High cell viability and increased mature myogenic marker expression	Extrusion bioprinting	(Loi et al., 2024)
Skeletal	Cyclic strain	Laminin-coated elastic silicone membrane	C2C12 murine myoblasts	Uniaxial stretch using a StrexCell® STB-1400	Cyclic tensile loading promoted myotube hypertrophy, anabolic signaling, whereas stretch withdrawal induced atrophy, inflammatory and catabolic signaling	N.A.	(Kamal et al., 2024)
Skeletal	Electrical	2D culture	Human myogenic precursor cells (hMPCs)	Detachable biphasic electrical current microcurrent generating chamber with platinum-coated electrodes combined with anisotropic surface topography	Enhanced myogenic differentiation, aligned myotube formation, and improved muscle regeneration and angiogenesis after transplantation	N.A.	(Jun et al., 2022)
Skeletal	Electrical	Fibrin, Matrigel	Primary human skeletal muscle cells, human dermal fibroblasts	Electrical stimulation bioreactor with 5 mm polystyrene microwells containing parallel elastic wires and parallel carbon electrodes for stimulation	Increased myotube size, contractile force, fatigue resistance; and promoted a fast-to-slow muscle phenotype shift	N.A.	(Pallotta et al., 2024)
Skeletal	Electrical	Fibrin, Matrigel	Healthy and DMD immortalized human myoblasts	24-well stimulation system with graphite electrodes, a selector module for targeted well stimulation, and PDMS molds with vertical flexible pillars	Enabled high-throughput functional stimulation, increased twitch/tetanic responses with maturation, and identified contraction-induced DMD muscle damage phenotypes	N.A.	(Ruiz-Gutierrez et al., 2025)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Electrical	Fibrin, Geltrex	Primary immortalized human myoblasts, hiPSC	Micropost deflection platforms with needle electrodes	Optimized electrical stimulation parameters revealed maximal contractile force and cell line-specific excitation-contraction properties of engineered muscle microtissues	N.A.	(Tiper et al., 2025)
Skeletal	Perfusion	Collagen, Matrigel	C2C12 murine myoblasts	Perfusion bioreactor enabling *in situ* differentiation of engineered skeletal muscle tissues between pillars	Reduced inflammatory and atrophic signaling while maintaining skeletal muscle maturation	N.A.	(Rimington et al., 2019)
Skeletal	Perfusion	Collagen	C2C12 murine myoblasts, primary chick myoblasts, chicken fibroblast cell line	Hollow fiber bioreactor with uniformly distributed perfusable hollow fiber arrays and microfabricated anchoring structures, active bidirectional perfusion	Enhanced mature myogenic marker expression, sarcomere organization, myotube alignment, tissue contractility, reduced necrosis, and enabled centimeter-scale muscle tissue formation	N.A.	(Nie et al., 2025)
Skeletal	Multimodal	Fibrin, alginate-RGD	C2C12 murine myoblasts	Custom electro-mechanical bioreactor for strain-based and electrical stimulation of aligned rotary wet-spun core-shell muscle bundles	Electromechanical stimulation modulated myotube maturation as well as mature myogenic marker expression depending on the stimulation protocol	Extrusion bioprinting	(Celikkin et al., 2023)
Skeletal	Multimodal	Fibrin, Matrigel	Human skeletal muscle cells	Electromechanical dynamic weight-training bioreactor enabling isotonic and isometric contractions under electrical stimulation with adjustable loads	Weight training increased contractile force, myofiber alignment, myofiber diameter, glucose consumption and lactate production, whereas isotonic loading produced greater functional improvements than isometric loading	N.A.	(Jo et al., 2024)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Skeletal	Multimodal	Collagen, Matrigel	C2C12 murine myoblasts	Electromechanical co-stimulation system providing simultaneous electrical stimulation and dynamically adjustable mechanical resistance with real-time force monitoring	Electromechanical co-stimulation significantly enhanced myoblast fusion, sarcomere formation, mature myogenic marker expression, calcium handling, and contractile force compared with electrical or mechanical stimulation alone	N.A.	(Yang et al., 2024a)
Skeletal	Multimodal	2D monoculture	C2C12 murine myoblasts	Electromechanical bioreactor with stretchable conductive poly (pyrrole)-PDMS membrane enabling cyclic stretching and electrical stimulation	Cyclic stretching aligned myoblasts and promoted parallel myotube formation, while electrical stimulation enhanced myogenic gene expression and myotube formation. Combined stimulation produced synergistic improvements in myogenic differentiation and generated aligned myotubes	N.A.	(Hu et al., 2024)
Skeletal	Multimodal	GelMA, alginate dialdehyde, glass nanoparticles	C2C12 murine myoblasts	Perfusion bioreactor integrating perfusion, electrical stimulation, in-line pH/temperature sensing, and extrusion bioprinted scaffolds	Perfusion improved cell viability, spreading, myoblast alignment, supported early myotube formation	Extrusion bioprinting	(Sprenger et al., 2026)
Cardiac	Cyclic strain	Collagen	Embryonic chick cardiac myocytes, neonatal rat cardiac myocytes	Motorized stretch device for engineered heart tissue	Improved cardiomyocyte hypertrophy, alignment and contractile force generation	N.A.	(Fink et al., 2000)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Cyclic strain	Gelatin	Human pediatric heart cells	Bio-Stretch mechanical stimulation apparatus	Improved cardiomyocyte proliferation, distribution, and scaffold strength	N.A.	(Akhyari et al., 2002)
Cardiac	Cyclic strain	Fibrin	Neonatal rat cardiomyocytes	Cyclic strain bioreactor using a voice-coil actuator	Increased contractile force, cardiomyocyte alignment, gap junction formation	N.A.	(Salazar et al., 2015)
Cardiac	Cyclic strain	Decellularized human skin scaffold	Human cardiac progenitor cells (hCPCs), hCPC-derived early cardiomyocytes	Compact tunable stretch bioreactor	Improved marker expression towards mature cardiomyocytes	N.A.	(Putame et al., 2020)
Cardiac	Cyclic strain	Ring-shaped fibrin scaffolds	Neonatal rat cardiomyocytes, neonatal rat fibroblasts	Uniaxial stretch bioreactor within situ non-destructive passive force monitoring	Improved cardiomyocyte alignment; gap junction formation, contractile force, and electrical responsiveness	N.A.	(Massai et al., 2020)
Cardiac	Perfusion	Polyglycolic acid	Neonatal rat cardiomyocytes	Perfusion bioreactor with direct medium flow through constructs	Improved cell distribution and expression of cardiac-specific markers	N.A.	(Carrier et al., 2002)
Cardiac	Perfusion	Rat aorta	Neonatal rat cardiomyocytes	Pulsatile perfusion bioreactor using a natural core vessel	Improved cell viability and metabolic activity	N.A.	(Kofidis et al., 2003)
Cardiac	Perfusion	Collagen	Neonatal rat cardiomyocytes	Perfusion cartridge system with interstitial medium flow through collagen constructs	Improved cell viability, density, and contractile function and recovery	N.A.	(Radisic et al., 2004b)
Cardiac	Perfusion	Collagen	Neonatal rat cardiomyocytes	Static culture with superficial medium flow during oxygen profiling	Improved oxygen availability within constructs	N.A.	(Radisic et al., 2005)
Cardiac	Perfusion	Collagen	Neonatal rat cardiomyocytes	Pulsatile perfusion bioreactor with flow through tissue membrane	Improved contractile function and hypertrophic maturation	N.A.	(Brown et al., 2008)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Perfusion	Gelatin	Human mesenchymal stem cells (hMSCs), human cardiac progenitor cells (hCMPCs)	Pulsatile perfusion bioreactor enabling continuous scaffold perfusion	Improved cell infiltration and cardiac and endothelial commitment, enabling prevascularized cardiac tissue formation	N.A.	(Pagliari et al., 2014)
Cardiac	Perfusion	Acellular porcine cardiac ventricular with preserved vascular tree	hMSCs, HUVEC-GFPs, hESC-derived cardiomyocytes	Perfusion bioreactor with physiological-like vascular perfusion and compartmentalized culture	Improved cell penetration, vascularization and generation of thick cardiac constructs	N.A.	(Sarig et al., 2015)
Cardiac	Perfusion	Compressed collagen scaffold with central perfusable channel	Human atrial appendage endothelial cells (HAAECs), human dermal fibroblasts (HDFs), hiPSC-derived cardiomyocytes/ pericytes	Perfusion bioreactor for scaffold fabrication and endothelialized channel culture	Improved prevascularized cardiac patch formation with an endothelialized perfusable channel and increased scaffold stiffness	N.A.	(Krziminski et al., 2020)
Cardiac	Electrical	Collagen	Neonatal rat myocytes	Electrical-stimulation bioreactor comparing electrode materials and stimulation regimes	Identified optimal stimulation conditions (3 V/cm, 3 Hz) improved tissue density, gap junction formation, and electrical responsiveness	N.A.	(Tandon et al., 2011)
Cardiac	Electrical	Fibrin	Neonatal rat myocytes	Petri-dish-based biphasic electrical stimulation chamber with carbon rods with the construct suspended between silicone posts	Improved cardiomyocyte elongation, gap junction formation, and electrical responsiveness	N.A.	(Lasher et al., 2012)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Electrical	Collagen	hESC-derived and hiPSC-derived cardiomyocytes with supporting fibroblasts, endothelial cells and smooth muscle cells	Electrical stimulation chamber combined with microfabricated tissues	Improved myofibril organization, conduction velocity, calcium handling, and electrical responsiveness	N.A.	(Nunes et al., 2013)
Cardiac	Electrical	Fibrin	Neonatal rat cardiomyocytes, hiPSC-derived cardiomyocytes	Biphasic electrical stimulation of engineered heart tissue	Improved cardiomyocyte elongation, gap junction formation, sarcomere organization, calcium handling, and contractile force generation	N.A.	(Hirt et al., 2014)
Cardiac	Electrical	2D culture	Neonatal rat cardiomyocytes	Electrical stimulator with carbon rod electrodes	Improved sarcomere organization, gap junction formation, and electrical responsiveness under charge-balanced biphasic stimulation	N.A.	(Gabetti et al., 2023)
Cardiac	Electrical	Collagen	Human iPSC-derived ventricular cardiomyocytes, human cardiac fibroblasts	Heart-on-chip platform integrating electrical pacing, conductive hydrogel pillar electrodes, optical force tracking and real-time field-potential recording	Improved sarcomere organization, gap junction formation, calcium handling, contractile force generation, and electrical responsiveness	N.A.	(Zhang et al., 2023)
Cardiac	Electrical	2D culture (monolayers and spheroids)	hiPSC-derived cardiomyocytes	Electrical stimulation bioreactor; includes modeled low-shear flow for medium exchange	Improved sarcomere organization, gap junction formation, and electrical responsiveness	N.A.	(Licata et al., 2025)
Cardiac	Electrical	2D and 3D fibrin culture	Neonatal rat cardiomyocytes	Symmetric, asymmetric and monophasic and biphasic stimulation with separately tunable half waves using carbon rod electrodes	Asymmetric biphasic stimulation improved calcium handling, electrical responsiveness, and a metabolic shift towards mature cardiomyocytes	N.A.	(Sileo et al., 2025)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Electrical	2D culture	Neonatal rat cardiomyocytes, neonatal rat ventricular fibroblasts	Electrical stimulation bioreactor with carbon electrodes and compatibility with a 12-well plate	Improved sarcomere organization, gap junction formation, and marker expression corresponding to electrical responsiveness	N.A.	(Cook et al., 2026)
Cardiac	Multimodal	Alginate	Neonatal rat cardiomyocytes	Perfusion chamber with integrated carbon electrodes	Increased cell elongation, sarcomere organization, and gap junction formation	N.A.	(Barash et al., 2010)
Cardiac	Multimodal	Collagen	Neonatal rat cardiomyocytes	Bioreactor enabling cyclic uniaxial stretch, real-time force measurement, electrical stimulation, perfusion and optical monitoring	Improved cell alignment, mature marker expression, and contractile force generation	N.A.	(Kensah et al., 2011)
Cardiac	Multimodal	Poly (glycerol sebacate)	Neonatal rat cardiomyocytes	Bioreactor integrating perfusion, electrical stimulation and unconstrained tissue contraction	Improved cell distribution and expression of cardiac-specific markers, and contractile properties	N.A.	(Maidhof et al., 2012)
Cardiac	Multimodal	RGD-modified alginate	Neonatal rat cardiac cells	Compression bioreactor with perfusion	Intermittent compression combined with perfusion improved cell elongation, sarcomere organization, gap junction formation. Continuous cyclic compression was detrimental	N.A.	(Shachar et al., 2012)
Cardiac	Multimodal	2D culture on PDMS	Human umbilical vein endothelial cells, human atrial fibroblasts and adult rat cardiomyocytes	Bioreactor with cyclic stretch, electric field stimulation and perfusion	Improved electrical responsiveness	N.A.	(Lu et al., 2013)
Cardiac	Multimodal	Porcine-based acellular myocardial scaffold	Rat mesenchymal stem cells	Bioreactor for coordinated biaxial mechanical and electrical conditioning	Improved cell alignment, expression of cardiac-specific markers, gap junction formation	N.A.	(Wang et al., 2013)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Multimodal	Fibrin	Neonatal rat cardiac cells	Electromechanical bioreactor with custom distention setup and carbon electrodes	Delayed electrical stimulation following mechanical stimulation showed the highest improvement in contractile force generation, and expression of mature markers	N.A.	(Morgan and Black, 2014)
Cardiac	Multimodal	Collagen	Human iPSC derived cardiomyocytes	Mechanical loading and electrical pacing system	Static stress improved cell alignment, contractile force generation, and tensile stiffness, whereas static stress combined with electrical pacing further increased contractile force generation and the expression of mature markers	N.A.	(Ruan et al., 2016)
Cardiac	Multimodal	Decellularized porcine small intestinal submucosa (SIS) or Biological Vascularized Matrix (BioVaM)	Neonatal rat cardiomyocytes, neonatal rat heart cells	Bioreactor combining controlled uniaxial stretch and matrix perfusion for large-scale patches	Improved cardiomyocyte alignment, contractility and endothelial network formation	N.A.	(Lux et al., 2016)
Cardiac	Multimodal	3D cardiac patch/ scaffold system	Neonatal rat cardiomyocytes, neonatal rat cardiac fibroblasts	Bioreactor chamber with bidirectional perfusion, electrical stimulation, optical monitoring and contractility testing	Combined perfusion and electrical stimulation improved cell viability, contractile properties, electrical responsiveness, and expression of mature markers	N.A.	(Visone et al., 2018)
Cardiac	Multimodal	Polyurethane	Human umbilical cord-derived mesenchymal stem cells (MSCs)	Bioreactor with synchronized pulsatile perfusion and electrical stimulation via pacemaker	Maintained cell viability and structural integrity under dual stimulation	N.A.	(Herrmann et al., 2019)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Cardiac	Multimodal	Fibrin	Neonatal rat cardiomyocytes, hiPSC-derived cardiomyocytes	Auxotonic and isotonic loading system with real-time force monitoring and integrated electrical stimulation capability	Improved cardiomyocyte alignment, sarcomere organization, contractile force, electrical functionality	N.A.	(Pisanu et al., 2022)
Cardiac	Multimodal	Porcine cardiac extracellular matrix	Bone marrow primary human MSCs	Perfusion bioreactor with synchronized action-potential-shaped electrical stimulation and physiological left-ventricle-like mechanical loading	Improved cellular proliferation and expression of early cardiac progenitor markers	N.A.	(Markish et al., 2025)
Cardiac	Multimodal	Fibrin hydrogel with perfusable channels	Neonatal rat cardiac cells	Perfusion bioreactor with integrated electrical stimulation	Perfusion promoted cardiomyocyte elongation. Electrical stimulation promoted cardiomyocyte elongation, sarcomere organization, and maturation. Co-stimulation yielded the highest number of mature cardiomyocytes, contractile properties, and electrical responsiveness	N.A.	(Reid et al., 2026)
Smooth	Cyclic strain	Collagen tubular scaffold	HUASMCs	Dual-mode fabrication and stretch bioreactor	Promoted contractile phenotype, cell distribution, and circumferential alignment	N.A.	(Bono et al., 2017)
Smooth	Cyclic strain	Collagen	Primary murine aortic vascular smooth muscle cells (VSMCs)	Custom computer-controlled biaxial bioreactor enabling cyclic equibiaxial and strip-biaxial loading with force measurement	Maintained mechanical homeostasis, force generation, and adaptive stiffness regulation	N.A.	(Paukner et al., 2024)
Smooth	Electrical	Decellularized sheep carotid artery	A-10 VSMCs	24-well plate with stainless steel screw electrodes	Promoted contractile phenotype, cell alignment, and reduced proliferation	N.A.	(Derhambakhsh et al., 2023)

(*Continued*)

TABLE 3 Continued

Tissue	Bioreactor type	Material(s)	Cell type(s)	Key structural feature(s)	Major findings	Biofabrication	References
Smooth	Perfusion	Fibrin-based 3-layer vascular graft	HUVECs, ASCs, SMCs	Pulsatile perfusion bioreactor for grafts	Promoted layer-specific alignment, contractile phenotype, and vascular network formation	N.A.	(Helms et al., 2021)
Smooth	Perfusion	PDMS membrane	Human aortic SMCs, Human aortic ECs	Pulsatile flow system	Maintained vascular cell alignment and physiological morphology under pulsatile flow	N.A.	(Meki et al., 2023)
Smooth	Perfusion	PVDF, fibrin, superparamagnetic iron oxide nanoparticles, PLGA, 19F-TPU	MSCs, HUASMCs, HUAECs	Pulsatile bioreactor with sensors	Promoted contractile phenotype, ECM deposition, and graft maturation	N.A.	(Rama et al., 2024)
Smooth	Perfusion	Decellularized plant scaffold	Primary rat ECs and SMCs	Closed-loop pulsatile perfusion	Improved endothelialization, reduced thrombogenicity, and maintained SMC viability	N.A.	(Gorbenko et al., 2024)
Smooth	Perfusion	Melt electrowritten PCL vascular graft	Human aortic SMCs, placental MSCs, ECFCs	Pulsatile perfusion bioreactor with physiological flow for vascular conditioning	Enhanced ECM deposition, graft strength, and cellularization	N.A.	(Weekes et al., 2025)
Smooth	Multimodal	PDMS membrane	hMSCs	Closed-loop perfusion and mechanical peristaltic stimulation using a rotating screw mechanism	Promoted myogenic differentiation, contractile phenotype, and cytoskeletal remodeling	N.A.	(Clevenger et al., 2023)

Abbreviations: 19F-TPU, fluorinated thermoplastic polyurethane; ASCs, adipose-derived stem cells; BioVaM, Biological Vascularized Matrix; dECM, decellularized extracellular matrix; DMD, Duchenne muscular dystrophy; ECFCs, endothelial colony-forming cells; ECs, endothelial cells; ECM, extracellular matrix; FSHD, facioscapulohumeral muscular dystrophy; GelMA, gelatin methacryloyl; GFP, green fluorescent protein; HAAECs, human atrial appendage endothelial cells; hCMPCs, human cardiac mesenchymal progenitor cells; hCPCs, human cardiac progenitor cells; HDFs, human dermal fibroblasts; hESCs, human embryonic stem cells; hiPSCs, human induced pluripotent stem cells; hMPCs, human myogenic precursor cells; hMSCs, human mesenchymal stem cells; HSkMC, human skeletal muscle cells; HUAECs, human aortic endothelial cells; HUASMCs, human aortic smooth muscle cells; HUVECs, human umbilical vein endothelial cells; MSCs, mesenchymal stem cells; PCL, polycaprolactone; PDMS, polydimethylsiloxane; PLGA, poly (D,L-lactide-co-glycolide); PNIPAM, poly (N-isopropylacrylamide); PVDF, poly (vinylidene fluoride); RGD, Arg-Gly-Asp peptide motif; SIS, small intestinal submucosa; SMCs, smooth muscle cells; VSMCs, vascular smooth muscle cells.

based platforms have become widely used for disease modelling, regenerative studies, and pharmacological screening (Madden et al., 2015; Tiburcy et al., 2019; Tejedera-Villafranca et al., 2023; Franken et al., 2025).

A second strategy has been the use of cyclic, or dynamic, uniaxial strain which more closely recapitulates the repeated loading and unloading experienced by native skeletal muscle during movement. Cyclic uniaxial loading has been associated with enhanced myogenic differentiation, increased expression of myogenic markers, improved myotube alignment, and greater functional maturation compared with static culture conditions (Aguilar-Agon et al., 2019; Tomasch et al., 2023; Kamal et al., 2024; Loi et al., 2024). In addition, cyclic loading can activate anabolic signaling pathways and promote muscle hypertrophy, indicating that dynamic mechanical cues contribute not only to structural organization but also to functional tissue development (Aguilar-Agon et al., 2019; Kamal et al., 2024).

Direct comparisons between static and cyclic mechanical loading strategies remain scarce. Collectively, current studies suggest that static tension effectively promotes tissue organization, alignment, and contractile function, whereas cyclic loading may provide additional benefits through hypertrophic and anabolic adaptations. However, cyclic loading typically requires

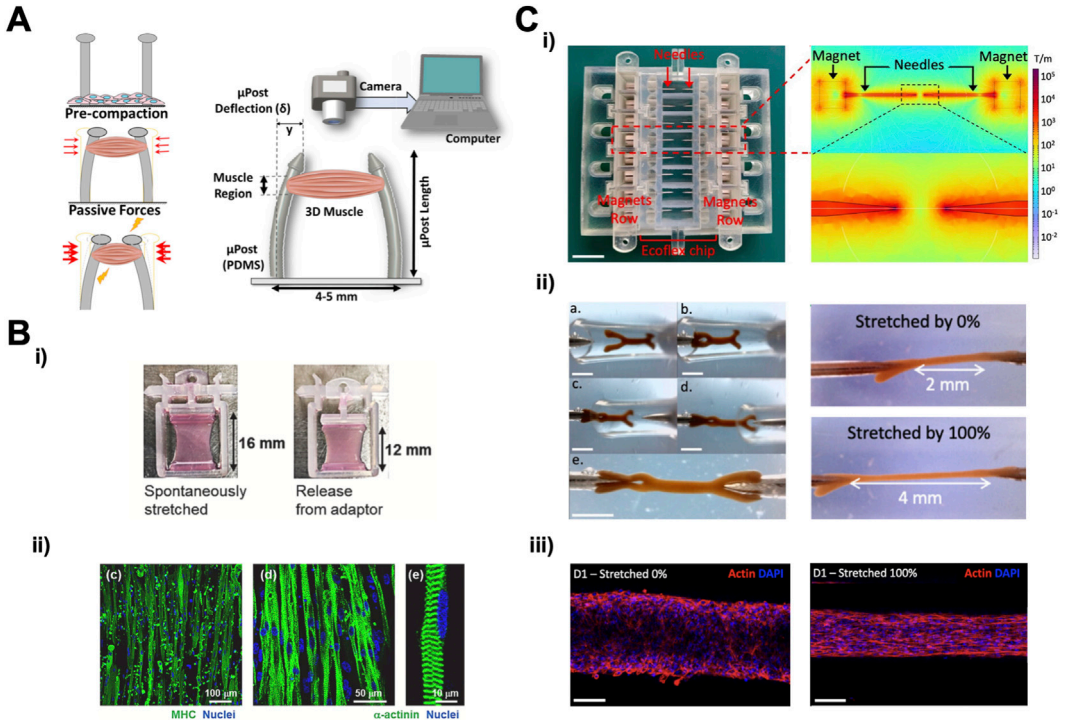

FIGURE 8
Overview of mechanical stimulation bioreactors for muscle tissue engineering. **(A)** Micropost-based force measurement platform in which muscle contraction induces post deflection, enabling quantification of contractile force. Adapted from (Vesga-Castro et al., 2022), CC BY 4.0. **(B)** Hanging-anchor tissue engineering approach. (i) Fibrin-based muscle constructs before and after release from the hanging adaptor. (ii) Fluorescence images showing aligned and differentiated myofibers after culture. Adapted from (Yoshida et al., 2025), CC BY 4.0. **(C)** Magnetically actuated stretching platform for engineered skeletal muscle tissues. (i) Magnetic needle array and corresponding magnetic field distribution. (ii) Tissue trapping and fixation procedure between magnetic needles. (iii) Enhanced tissue anisotropy and myogenic maturation following mechanical stretching. Adapted from (Demri et al., 2026b), CC BY 4.0.

more complex hardware and careful optimization of stimulation parameters. Furthermore, relatively few studies have systematically combined mechanical conditioning with biofabrication strategies that independently introduce architectural anisotropy at the scaffold level. Consequently, the relative contributions of scaffold architecture, static tension, and cyclic strain to skeletal muscle maturation remain unclear, highlighting the need for systematic comparative studies.

4.1.2 Mechanical stimulation of cardiac muscle

Mechanical loading is a fundamental regulator of cardiac development, maturation, and function, as the myocardium continuously experiences cyclic stretch and contraction throughout the cardiac cycle. These biomechanical cues regulate cardiomyocyte alignment, sarcomere organization, extracellular matrix remodeling, and excitation–contraction coupling through mechanotransduction pathways, making mechanical stimulation (see Figure 9 and Table 3) an essential component of engineered cardiac tissue maturation (Stoppel et al., 2016).

One widely adopted strategy is the use of auxotonic loading of engineered cardiac tissues, which promote structural and functional maturation while simultaneously enabling assessment of contractile force generation. Auxotonic loading is typically achieved by suspending engineered cardiac tissues between flexible posts, where tissue contraction induces post deflection and generates a mechanical load proportional to the contractile force produced by the tissue itself (Liaw and Zimmermann, 2016). Such loading conditions have been shown to enhance structural organization, cardiomyocyte elongation and alignment, sarcomere organization, improved excitation responsiveness, and increased contractile force generation (Kah et al., 2025). Furthermore, the mechanical properties of the support structures strongly influence tissue development, as post stiffness affects cardiomyocyte morphology, differentiation, and force production (Kah et al., 2025).

Another stimulation approach involves the use of cyclic mechanical stretch to reproduce the repetitive contractions of the native myocardium. Dynamic mechanical loading has been associated with increased cardiomyocyte alignment, improved structural organization, enhanced cardiac marker expression, tissue compaction and remodeling, increased tensile strength, and improved pacing-responsive contractility (Fink et al., 2000; Akhyari et al., 2002; Salazar et al., 2015; Lux et al., 2016; Massai et al., 2020; Putame et al., 2020). These findings indicate that mechanical stimulation contributes not only to structural organization but also to the functional maturation of engineered cardiac tissues.

Together, auxotonic and cyclic loading strategies demonstrate that mechanical conditioning promotes both structural and functional maturation of engineered cardiac tissues. Notably, the effectiveness of these approaches is strongly influenced by intrinsic construct properties, such as scaffold stiffness, architecture, and anisotropic organization, underscoring the close relationship

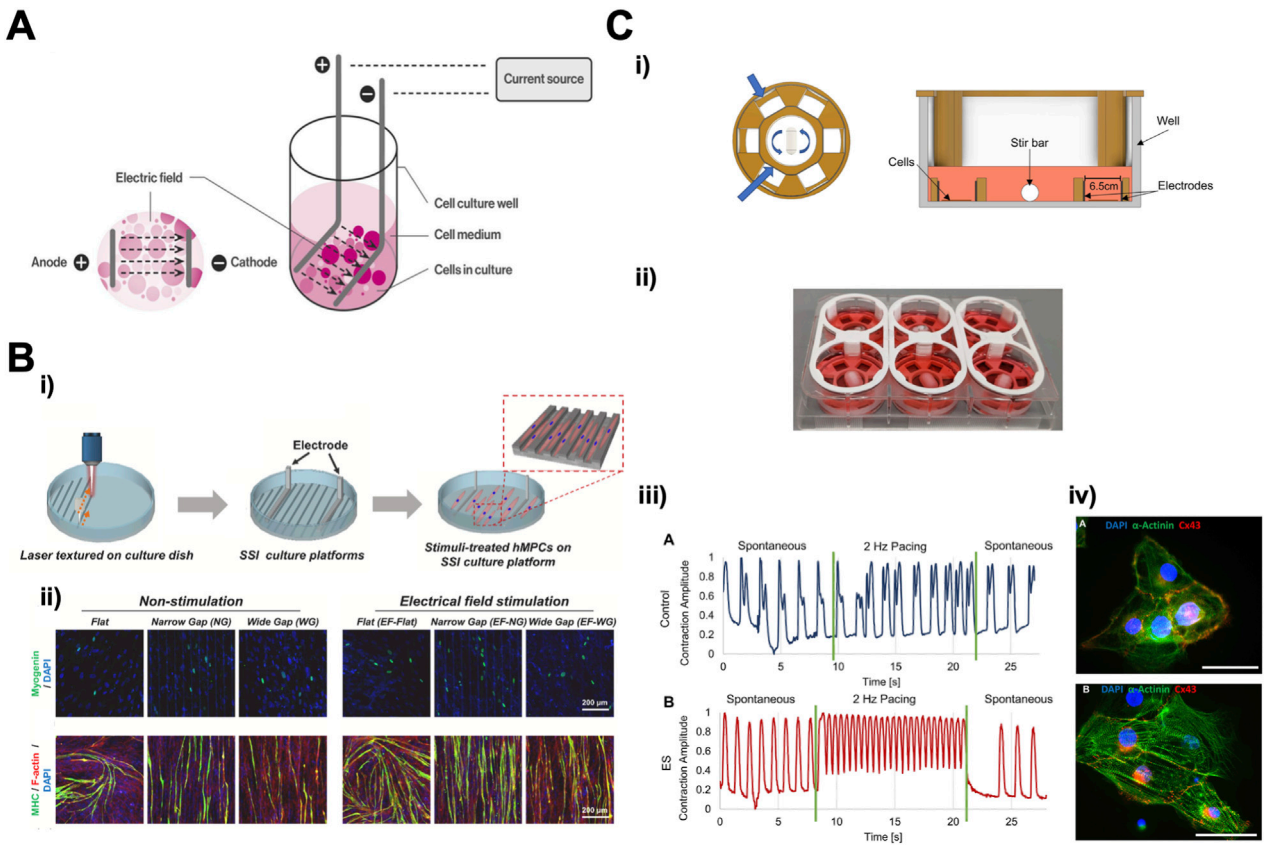

FIGURE 9
Overview of electrical pulse stimulation bioreactors for muscle tissue engineering. **(A)** Schematic representation of a conventional electric field stimulation setup. Adapted from (Ryan et al., 2021), CC BY 4.0. **(B)** Electrical stimulation combined with anisotropic culture substrates. (i) Simultaneous stimulation induction (SSI) platform integrating surface topography and electrical cues. (ii) Representative immunofluorescence images showing myogenic differentiation and myotube maturation of human muscle precursor cells cultured on SSI platforms under different topographical and electrical stimulation conditions. Adapted from (Jun et al., 2022), CC BY 4.0. **(C)** Electrically stimulated bioreactor platform. (i) CAD rendering and cross-sectional schematic of the custom bioreactor with integrated electrodes and stirring system. (ii) Photograph of six assembled bioreactors in a six-well plate. (iii) Representative cardiomyocyte contraction recordings before, during, and after electrical pacing. (iv) Immunofluorescence images demonstrating enhanced cardiomyocyte maturation following electrical stimulation, stained for α-actinin, Connexin43, and DAPI. Adapted from (Licata et al., 2025), CC BY 4.0.

between biofabrication and bioreactor-based conditioning strategies. Although such integrated approaches remain uncommon, recent studies have combined 3D bioprinting with auxotonic loading platforms, illustrating how tissue architecture and mechanical conditioning can be co-engineered to promote maturation and functional assessment (Esser et al., 2023).

4.1.3 Mechanical stimulation of smooth muscle

Mechanical conditioning promotes smooth muscle maturation and maintenance of the contractile phenotype by recapitulating key aspects of the native mechanical environment (see Table 3). In the absence of appropriate mechanical cues, cultured smooth muscle cells often transition toward a synthetic phenotype characterized by proliferation and extracellular matrix production rather than the contractile phenotype observed in native tissues (Jensen et al., 2021).

Cyclic circumferential strain has been shown to promote circumferential cell alignment, improve cell distribution throughout engineered constructs, and support maintenance of the contractile phenotype compared with static culture conditions (Bono et al., 2017). Consistent with this concept, biaxial mechanical conditioning has proven to improve elastic fiber maturation, collagen organization, compliance, and mechanical strength compared with conventional circumferential stimulation alone (Huang et al., 2016). Recent studies using multiaxial loading bioreactors suggest that vascular smooth muscle cells actively regulate their force-generating behavior in response to changes in mechanical loading, supporting the concept of mechanical homeostasis (Paukner et al., 2024).

Together, these findings demonstrate that mechanical conditioning is critical for maintaining the contractile smooth muscle phenotype and promoting functional tissue development. Future advances will likely depend on combining mechanical stimulation with biofabrication strategies that provide precise control over cell alignment and tissue architecture.

4.2 Electrical stimulation

Electrical stimulation, also termed electrical pulse stimulation, is employed to mimic the electrophysiological activity that regulates

muscle function *in vivo* (Ryan et al., 2021). In addition to inducing contraction, electrical stimulation can promote cellular maturation, improve tissue organization, and enhance functional performance in engineered muscle (Bravo-Olin et al., 2024; Williamson et al., 2024). Electric field stimulation is typically delivered using monophasic or biphasic pulse waveforms, with the choice of stimulation parameters depending on the muscle type and intended biological response (Alsenaide et al., 2025).

However, the biological effects of electrical stimulation are highly dependent on stimulation parameters and electrode configuration, requiring careful optimization to maximize efficacy while minimizing adverse electrochemical effects (Alsenaide et al., 2025).

4.2.1 Electrical stimulation of skeletal muscle

Since skeletal muscle is an excitable tissue, *in vitro* electric field stimulation (see Table 3) aims to replicate aspects of motor neuron-induced excitation-contractions and has therefore emerged as an important maturation strategy for engineering skeletal muscle tissues (Vesga-Castro et al., 2022). Importantly, emerging evidence suggests that the effectiveness of electrical stimulation is strongly influenced by tissue architecture and material design, highlighting the close interaction between biofabrication and bioreactor-based conditioning strategies. Studies combining electrical stimulation with anisotropic scaffold architectures or conductive biomaterials reported synergistic improvements in myotube alignment, fusion, maturation and functional performance compared with either strategy alone (Jun et al., 2022; Xue et al., 2024).

Electrical stimulation has been shown to promote myotube alignment, sarcomere organization, muscle hypertrophy, and functional maturation, resulting in increased force production, and enhanced fatigue resistance compared with unstimulated controls (Lee et al., 2022; Pallotta et al., 2024). Furthermore, electrical stimulation partially recapitulates exercise-associated signaling pathways, leading to increased myotube size, improved contractile performance, and exercise-like adaptations in muscle phenotype (Pallotta et al., 2024).

Low-frequency stimulation produces individual twitch contractions, whereas increasing stimulation frequency induces tetanic contractions characterized by sustained force generation. Because tetanic contractions more closely reflect the maximal force-generating capacity of engineered muscle tissues, tetanic force and the tetanic-to-twitch ratio are frequently used as indicators of tissue maturation, contractile performance, and fatigue resistance (Vesga-Castro et al., 2022; Pallotta et al., 2024; Ruiz-Gutierrez et al., 2025; Tiper et al., 2025). Consequently, stimulation parameters including frequency, pulse duration, field strength, and waveform characteristics must be carefully optimized according to the cell source, tissue architecture, and intended application (Tiper et al., 2025; van de Meene et al., 2025). Beyond its role as a maturation stimulus, electrical stimulation has become a valuable tool for disease modelling, functional phenotyping, and therapeutic screening in engineered skeletal muscle tissues (Tejedera-Villafranca et al., 2023; Ruiz-Gutierrez et al., 2025).

Together, these findings demonstrate that electrical stimulation serves not only as a maturation cue but also as a tool for functional characterization of engineered skeletal muscle. However, the interactions between stimulation protocols, tissue architecture, and biofabrication strategies remain incompletely understood. Future studies should systematically evaluate how electrical conditioning interacts with construct anisotropy, cellular organization, and conductive biomaterials to maximize tissue maturation and functionality.

4.2.2 Electrical stimulation of cardiac muscle

Electrical stimulation is a widely used maturation strategy in cardiac tissue engineering because electrical signaling plays a central role in cardiac development, excitation-contraction coupling, and the synchronized contraction of native myocardium. Reproducing these electrical cues *in vitro* is therefore considered essential for the development of functionally mature engineered cardiac tissue. Because engineered cardiac tissues often exhibit immature electrophysiological properties and irregular spontaneous beating, electrical stimulation has become a widely used strategy to promote functional maturation *in vitro* (see Figure 9 and Table 3) (Tandon et al., 2009; Tandon et al., 2011; Stoppel et al., 2016).

Establishing coordinated excitation and contraction at the tissue level is considered a major indicator of functional maturation in engineered cardiac tissue, reflecting improved electrical coupling and integration of cardiomyocytes into a functional syncytium (Vunjak-Novakovic et al., 2010; Weinberger et al., 2017). Accordingly, electric field stimulation has been shown to promote cardiomyocyte alignment and coupling, sarcomere organization, connexin-43 expression, conduction velocity, and improved contractile performance, while reducing excitation thresholds and improving pacing responsiveness (Radisic et al., 2004a; Lasher et al., 2012; Nunes et al., 2013; Hirt et al., 2014; Gabetti et al., 2023; Licata et al., 2025). These findings demonstrate that electric field stimulation promotes both structural and functional maturation of engineered cardiac muscle.

Multiple studies further demonstrated that stimulation outcomes depend strongly on field strength, pulse duration, pacing frequency, electrode material, and waveform characteristics (Tandon et al., 2009; Tandon et al., 2011). Alternative waveform designs include charge-balanced biphasic and asymmetric biphasic waveforms, which can improve maturation while reducing charge accumulation and electrode-associate side effects (Gabetti et al., 2023; Sileo et al., 2025). These findings highlight that the biological effects of electrical stimulation depend not only on the presence of pacing but also on the delivery of electrical cues.

Together, these studies demonstrate that electrical stimulation is more than a pacing tool. By promoting synchronized electrical activity, enhancing excitation-contraction coupling, and driving structural and electrophysiological maturation, electrical stimulation has been widely used in cardiac tissue engineering and is increasingly incorporated into multimodal bioreactor platforms that combine electrical, mechanical, and perfusion-related cues.

4.2.3 Electrical stimulation of smooth muscle

Compared with skeletal and cardiac muscle tissue engineering, the application of electric field stimulation in smooth muscle tissue

engineering remains relatively limited (see Table 3). This may partly reflect the diversity of smooth muscle electrophysiology across different organs, where electrical activity is influenced by syncytial organization, distributed innervation, and tissue-specific physiological functions (Manchanda et al., 2019).

Nevertheless, electrical stimulation has demonstrated potential for regulating smooth muscle cell behavior and phenotype. In vascular smooth muscle cell constructs, electrical stimulation promoted a shift from a synthetic toward a contractile phenotype, characterized by elongation of the cells, decreased expression of synthetic phenotype markers, and increased expression of the contractile markers α-SMA and SMMHC (Derhambakhsh et al., 2023).

These findings demonstrate that electrical stimulation can influence smooth muscle phenotype and function. Although electrical stimulation shows promise for regulating smooth muscle phenotype and function, further research is required to establish optimal stimulation protocols. Given the diversity of smooth muscle tissues and their electrophysiological characteristics across different organs, these protocols will likely need to be tailored to specific applications. However, the limited number of studies highlights the need for further investigation, particularly to establish organ-specific stimulation protocols and to determine how electrical cues can be integrated with biofabrication and other bioreactor-based conditioning strategies.

4.3 Perfusion

One of the major challenges in muscle tissue engineering is the generation of clinically relevant tissue volumes while maintaining high cell viability throughout the construct. As construct dimensions increase, passive diffusion becomes insufficient to support oxygen and nutrient transport, resulting in the formation of hypoxic regions, reduced tissue viability, and impaired tissue development (Yang et al., 2020; Wang et al., 2023). Perfusion bioreactors aim to address these limitations by driving culture medium through or around engineered tissues, thereby enhancing oxygen and nutrient delivery while facilitating waste removal (Bravo-Olin et al., 2024; Williamson et al., 2024).

Biofabrication strategies have also aimed to improve mass transport through the incorporation of porous architectures, perfusable microchannels, and vascular-like networks (Schätzlein and Blaeser, 2022; Liu et al., 2026). Perfusion bioreactors can complement such biofabricated designs by actively driving medium through engineered transport pathways, thereby supporting the long-term culture and maturation of larger and more complex muscle tissues (Bravo-Olin et al., 2024; Williamson et al., 2024; Cordelle et al., 2025).

4.3.1 Perfusion for skeletal muscle

As skeletal muscle tissue engineering advances toward clinically relevant volumetric muscle constructs and volumetric muscle loss applications, diffusion-mediated transport becomes a major limitation. Perfusion bioreactors have therefore been investigated to enhance oxygen and nutrient delivery while facilitating waste removal throughout larger three-dimensional tissues (see Table 3) (Gholobova et al., 2020; Gilbert-Honick and Grayson, 2020).

Perfusion culture has been associated with improved cell distribution, enhanced viability, reduced transport limitations, and increased myogenic maturation of skeletal muscle constructs (Cerino et al., 2016; Nie et al., 2025). Continuous perfusion has furthermore been shown to reduce inflammatory and catabolic signaling while supporting myotube development and tissue maturation (Rimington et al., 2019).

These findings demonstrate that perfusion bioreactors can overcome mass transport limitations while supporting skeletal muscle maturation and functionality. As advances in biofabrication enable the generation of larger and more complex skeletal muscle constructs, the incorporation of perfusion systems and perfusable architectures will likely play an increasingly important role in overcoming transport limitations and sustaining tissue development.

4.3.2 Perfusion for cardiac muscle

Perfusion bioreactors have become an important strategy for engineering metabolically active cardiac tissues, as larger engineered cardiac constructs can be affected by insufficient oxygen and nutrient transport, compromising cell viability, maturation, and contractile performance over time (Bravo-Olin et al., 2024). Consequently, the development of strategies that support efficient mass transport and establish stable, functional perfusion remains a central challenge in cardiac tissue engineering (see Figure 10 and Table 3) (Liu et al., 2026).

Perfusion-based culture has been shown to improve cell density, viability, and metabolic activity of 3D cardiomyocyte-laden scaffolds, highlighting the role of oxygen and mass transport for the development of larger cardiac tissue constructs (Carrier et al., 2002; Kofidis et al., 2003; Radisic et al., 2005; Radisic et al., 2008). Other studies demonstrated that medium flow also supports the formation of contractile cardiac tissues, outperforming static culture conditions (Radisic et al., 2004b; Brown et al., 2008). These findings highlight the importance of perfusion on both cellular activity and functional cardiac tissue performance. More recently, perfusion has been integrated with vascularization strategies, including prevascularized constructs and endothelial cell-containing perfusable channels, enabling the fabrication of larger and more physiologically relevant cardiac tissues (Pagliari et al., 2014; Sarig et al., 2015; Krziminski et al., 2020).

Together, these advances demonstrate how perfusion strategies have evolved beyond simple nutrient-delivery systems into platforms for the development of vascularized and physiologically relevant engineered myocardium. In parallel, advances in biofabrication have enabled the generation of porous and perfusable cardiac constructs, providing new opportunities to integrate perfusion more effectively throughout engineered tissues and further improve oxygen delivery, vascularization, and long-term tissue function (Liu et al., 2026).

4.3.3 Perfusion for smooth muscle

Perfusion bioreactors are widely utilized bioreactor systems for smooth muscle tissue engineering. Pulsatile perfusion is commonly used to mimic physiological loading by reproducing vascular distension and peristaltic-like stimulation in visceral smooth

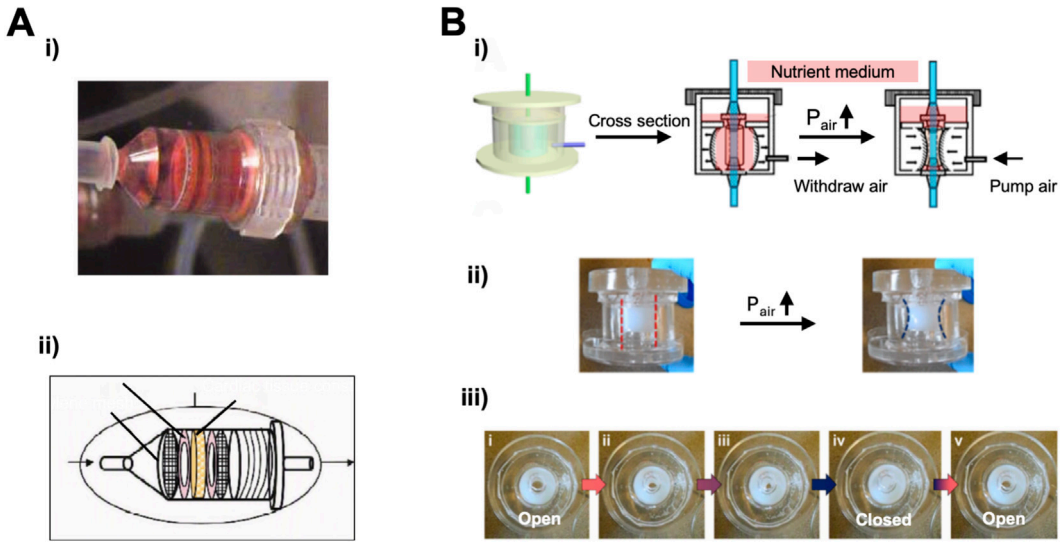

FIGURE 10
Overview of perfusion bioreactors for muscle tissue engineering. **(A)** Pulsatile perfusion bioreactor for cardiac tissue engineering. (i) Cardiac tissue construct housed within the perfusion chamber. (ii) Schematic of the perfusion cartridge showing the cardiac tissue construct positioned between silicone gaskets and polypropylene meshes. Adapted from (Brown et al., 2008), with permission. **(B)** Pneumatically actuated perfusion bioreactor. (i) Cross-sectional schematic of the bioreactor cartridge illustrating perfusion and air-driven deformation of the culture chamber. (ii) Side-view images demonstrating cartridge deformation under pneumatic actuation. (iii) Sequential top-view images showing cyclic opening and closing during operation. Adapted from (Zhou et al., 2018), CC BY 4.0.

muscle tissues (see Figure 10 and Table 3). Despite these tissue-specific differences, both approaches provide dynamic mechanical cues that regulate smooth muscle cell alignment, phenotype, and tissue organization (Helms et al., 2021).

Tissue-engineered vascular grafts represent one of the most extensively studied applications. Pulsatile perfusion has been shown to enhance cell viability, extracellular matrix deposition, collagen accumulation, burst pressure, and suture retention strength, while promoting circumferential smooth muscle cell alignment resembling the fiber orientation in native blood vessels (Helms et al., 2021; Meki et al., 2023; Gorbenko et al., 2024; Rama et al., 2024; Weekes et al., 2025). Furthermore, hemodynamic parameters including pressure, flow rate, pulsatility, and vessel geometry significantly influence tissue development and should therefore be carefully considered during bioreactor design (Bilodeau et al., 2005; Meki et al., 2023).

Together, these studies demonstrate that perfusion-based stimulation promotes smooth muscle tissue maturation. Future progress will likely depend on integrating perfusion bioreactors with biofabrication strategies that provide precise control over smooth muscle cell distribution and alignment to better recreate native tissue architecture.

4.4 Multimodal bioreactors

While conventional bioreactors typically focus on a single mode of stimulation, engineered muscle tissue development is regulated by a combination of biochemical, mechanical, and electrical cues that interact throughout culture. Consequently, bioreactor systems increasingly integrate multiple stimulation modalities and sensing technologies within a single platform.

Rather than applying isolated stimuli, multimodal bioreactors combine tissue conditioning strategies such as mechanical loading, electrical stimulation, and perfusion, sometimes in the presence of integrated monitoring systems. This enables simultaneous control of the culture environment and real-time evaluation of tissue development, maturation, and function (Williamson et al., 2024).

As biofabrication strategies continue to generate increasingly complex tissue constructs, multimodal bioreactors may offer additional opportunities to guide tissue development, optimize culture conditions, and monitor construct performance in real time.

4.4.1 Multimodal stimulation of skeletal muscle

Native skeletal muscle development and adaptation are regulated by the coordinated interaction of mechanical loading, electrical excitation, and biochemical transport processes. Consequently, bioreactors that integrate multiple forms of stimulation have emerged as a promising strategy to better recapitulate the physiological environment of skeletal muscle tissues (see Figure 11 and Table 3).

Studies combining electrical and mechanical stimulation have demonstrated that these cues can interact synergistically to promote skeletal muscle maturation. Electromechanical conditioning has been associated with enhanced myogenic gene expression, myotube formation, sarcomere organization, calcium handling, and contractile force generation, often outperforming either stimulus alone, suggesting that mechanical and electrical signals regulate complementary aspects of muscle development (Celikkin

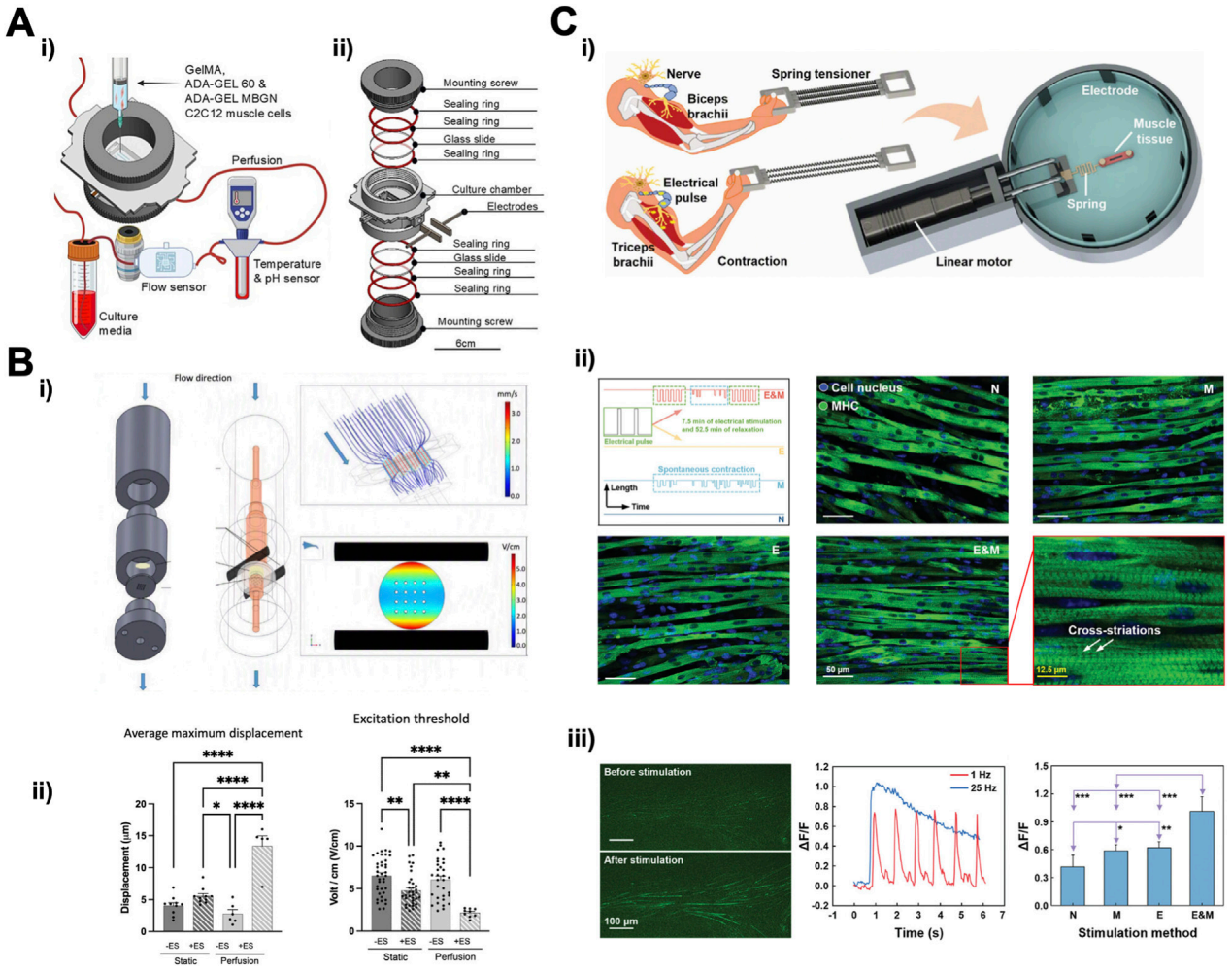

FIGURE 11
Overview of multimodal, multistimulation, and multisensing bioreactors for muscle tissue engineering. **(A)** Perfused bioreactor platform for dynamic culture of biofabricated skeletal muscle constructs. (i) Experimental workflow for culturing cell-laden bioinks under perfusion. (ii) Exploded CAD view of the bioreactor assembly. Adapted from (Sprenger et al., 2026), CC BY 4.0. **(B)** Perfusion-electrical stimulation bioreactor for engineered cardiac tissues. (i) Bioreactor design showing integrated perfusion, electrical stimulation, and simulated flow and electric field distributions. (ii) Functional assessment of cardiomyocyte-laden constructs comparing static and perfusion culture with or without electrical stimulation, demonstrating enhanced contractile activity and reduced excitation thresholds under combined stimulation conditions. Adapted from (Reid et al., 2026), CC BY 4.0. **(C)** Electromechanical co-stimulation platform inspired by skeletal muscle training. (i) Schematic of the electromechanical co-stimulation system integrating cyclic stretching and electrical stimulation. (ii) Structural maturation of engineered skeletal muscle tissues cultured under no stimulation (N), mechanical stimulation (M), electrical stimulation (E), or combined electromechanical stimulation (E&M), including the formation of cross-striations. (iii) Functional assessment of Ca2+ handling capacity, including representative fluorescence images, Ca2+ transient traces, and quantitative comparison of stimulation groups, demonstrating enhanced function following combined electromechanical stimulation. Adapted from (Yang L. et al., 2024), with permission.

et al., 2023; Hu et al., 2024; Yang L. et al., 2024). Exercise-inspired platforms further indicate that combining electrically induced contraction with mechanical resistance may provide a more physiologically relevant training environment, promoting structural, functional, and metabolic maturation of engineered skeletal muscle tissues (Jo et al., 2024).

These studies demonstrate a growing trend toward the integration of multiple stimulation modalities, sensing technologies, and functional readouts within a single culture platform. While several studies report enhanced maturation outcomes following combined stimulation strategies, further work is needed to establish whether multimodal systems provide reproducible and broadly applicable benefits beyond those achievable with optimized single-stimulus approaches.

4.4.2 Multimodal stimulation of cardiac muscle

For cardiac muscle, native myocardial maturation is governed by the coordinated interaction of electrical excitation, mechanical loading, perfusion, and continuous feedback between tissue function and the surrounding environment. While conventional single stimulus bioreactors can improve selected aspects of engineered cardiac tissue maturation, they only partially reproduce the integrated environment of the heart consisting of excitation, contraction, and perfusion. Consequently, cardiac bioreactor development has progressively shifted from single stimulation systems towards multimodal platforms that combine mechanical, electrical, and perfusion stimulation as well as real-time functional monitoring (see Figure 11 and Table 3).

Electric field stimulation has been among the most widely used single stimulation bioreactors for maturing engineered cardiac muscle due to its central role in cardiomyocyte maturation and synchronized contraction (Bravo-Olin et al., 2024). When combined with perfusion, electrical stimulation has been associated with improvements in tissue organization, contractile performance, cardiomyocyte maturation, and cardiac protein expression (Barash et al., 2010; Maidhof et al., 2012; Visone et al., 2018; Reid et al., 2026). In several studies, these outcomes exceeded those obtained with either stimulation modality alone (Maidhof et al., 2012; Reid et al., 2026). These synergistic effects likely arise because perfusion enhances oxygen and nutrient delivery throughout the construct, while electrical stimulation promotes structural and functional maturation.

Electromechanical bioreactors further extend this concept by combining controlled mechanical loading with electric field-induced pacing. Such systems have been associated with enhanced cardiomyocyte alignment, sarcomere organization, excitation-contraction coupling, force generation, and functional maturation (Wang et al., 2013; Morgan and Black, 2014; Ruan et al., 2016; Zhang et al., 2023). Importantly, several studies demonstrated that not only the presence but also the timing of electrical and mechanical cues influences maturation outcomes, highlighting the importance of recapitulating physiological excitation-contraction coupling *in vitro* (Morgan and Black, 2014). In addition, other cardiac bioreactors incorporated perfusion into electric field or mechanical stimulation bioreactors, thereby aiming to overcome mass transport limitations during culture (Lu et al., 2013; Markish et al., 2025).

An additional trend in cardiac bioreactor development has been the incorporation of sensing technologies for continuous, non-destructive assessment of tissue maturation. Examples include systems incorporating *in situ* force measurements, optical monitoring, electrophysiological recording, and contractility testing during culture (Kensah et al., 2011; Visone et al., 2018; Zhang et al., 2023). Such platforms not only provide maturation cues but also enable real-time evaluation of tissue development, creating opportunities for future feedback-controlled and adaptive conditioning strategies.

4.4.3 Multimodal stimulation of smooth muscle

Single-stimulus bioreactors, particularly perfusion-based systems, have been widely used for smooth muscle tissue engineering because they reproduce essential physiological cues such as nutrient transport, fluid flow, and shear stress (Helms et al., 2021; Clevenger et al., 2023; Weekes et al., 2025). Perfusion-based systems have also been adapted to mimic peristaltic environments, where dynamic stimulation alters cytoskeletal organization, proliferation, nuclear morphology, and smooth muscle-associated gene expression, indicating a role in myogenic maturation (Clevenger et al., 2023). Despite the widespread occurrence of smooth muscle tissues in the gastrointestinal, urinary, and reproductive systems, bioreactor studies investigating these applications remain relatively scarce compared with vascular tissue engineering.

However, native smooth muscle tissues are simultaneously exposed to multiple mechanical, biochemical, electrical, and hemodynamic cues that collectively regulate cellular alignment, tissue remodeling, contractility, and phenotypic plasticity. Reproducing these synergistic environmental cues remains a major challenge in smooth muscle tissue engineering (Gao, 2017; Baldwin et al., 2024).

Despite these complex *in vivo* conditions, the application of multimodal stimulation strategies in smooth muscle tissue engineering remains surprisingly limited (see Table 3). In contrast to skeletal and cardiac muscle tissue engineering, where integrated bioreactor platforms combining multiple conditioning modalities have become increasingly common, smooth muscle tissue engineering studies have largely focused on single-modality stimulation, leaving the synergistic or antagonistic interactions of combined stimuli largely unexplored.

Consequently, the individual and combined contributions of different biophysical cues to smooth muscle maturation, phenotype regulation, extracellular matrix remodeling, and functional tissue development remain poorly understood. Addressing this knowledge gap may be particularly relevant for vascular, gastrointestinal, urinary, and reproductive tissue engineering applications, where smooth muscle function depends on the coordinated integration of multiple environmental stimuli.

5 Biofabrication-bioreactor co-design considerations

Significant advances have been achieved in both biofabrication and bioreactor technologies for skeletal, cardiac, and smooth muscle tissue engineering. Increasingly sophisticated scaffold architectures, multimaterial bioinks, and multicellular constructs have enabled improved recapitulation of native muscle tissue organization and composition. Likewise, bioreactor-based conditioning has promoted tissue maturation through mechanical, electrical, perfusion-based, and multimodal stimulation strategies, sometimes combined with integrated sensing and functional assessment systems. Despite these developments, biofabrication and bioreactor technologies are still frequently optimized independently, creating critical mismatches between construct design and subsequent maturation requirements. Addressing these challenges will require construct-bioreactor co-design approaches in which fabrication and maturation are considered as interdependent components of a unified tissue engineering workflow rather than as sequential and independently optimized stages.

5.1 Scaling biofabricated tissues introduces mass transport challenges

As muscle tissue engineering progresses towards clinically relevant dimensions, increasingly larger and more complex constructs are being developed. However, scaling biofabricated tissues inevitably introduces mass transport limitations related to oxygen delivery, nutrient diffusion, and metabolic waste removal. This challenge is particularly relevant for volumetric skeletal muscle constructs, engineered myocardium, and vascularized smooth muscle tissues, where cell survival and function become increasingly dependent on efficient transport mechanisms.

Addressing these limitations through biofabrication may involve the incorporation of controlled porosity, perfusable channels, or

engineered vascular networks through multicellular and co-culture approaches (Lovett et al., 2009; Grebenyuk et al., 2023). In parallel, bioreactor-based strategies such as perfusion conditioning can actively support nutrient transport and improve environmental homogeneity throughout the construct (Filippi et al., 2023). While either approach may improve tissue viability independently, future muscle tissue models will likely require integrated solutions combining vascular biofabrication strategies with dynamic perfusion systems. Such synergistic approaches will likely become essential for maintaining cell viability and supporting functional maturation as engineered muscle tissues progress toward clinically relevant dimensions.

5.2 Static biomaterials versus dynamic maturation requirements

Biomaterials selected for optimal printability and cytocompatibility may not necessarily be compatible with long-term bioreactor-based conditioning. Bioinks are frequently optimized within the biofabrication window, balancing printing fidelity, structural integrity, and cell viability. However, following fabrication, engineered muscle tissues may be exposed to prolonged mechanical loading, electrical stimulation, perfusion, or combinations thereof.

Materials that perform well during fabrication may lack sufficient robustness to withstand repetitive cyclic loading, while mechanically stable biomaterials may restrict cell migration, extracellular matrix remodeling, or tissue development. Similarly, conductive biomaterials may improve electrical signal propagation and enhance tissue responsiveness to electrical conditioning, but can introduce additional considerations regarding degradation behavior, mechanical performance, and long-term biological functionality (Zhao et al., 2022; Zou et al., 2026).

Beyond static scaffold design, future biomaterials may increasingly be engineered to actively participate in the maturation process. Native muscle development is inherently dynamic, involving continuous changes in cellular organization, extracellular matrix composition, and tissue mechanics. In contrast, most biofabricated constructs possess largely fixed properties following fabrication. Emerging 4D biofabrication approaches aim to address this limitation through stimuli-responsive biomaterials capable of controlled changes in shape, stiffness, architecture, or biological functionality over time (Kalogeropoulou et al., 2024; Lai et al., 2024). Importantly, bioreactor-derived stimuli such as mechanical loading, electrical stimulation, perfusion, or biochemical cues may function not only as maturation factors but also as triggers for programmed material transformations, creating opportunities for dynamically adaptive tissue engineering systems. Such systems may enable staged tissue development in which scaffold properties evolve alongside changing cellular requirements, thereby providing a direct bridge between construct fabrication and bioreactor-guided maturation.

5.3 Immature tissues likely require stage-specific conditioning

A functional gap remains between engineered and native muscle tissues despite substantial progress in both biofabrication and bioreactor technologies. This challenge is particularly relevant for models relying on immature cell sources, including immortalized myoblast cell lines, neonatal cardiomyocytes, and stem-cell-derived muscle cells, which often exhibit phenotypic and functional characteristics distinct from their adult counterparts (Karbassi et al., 2020; Du et al., 2026).

Current bioreactor studies frequently apply fixed stimulation regimes throughout culture. However, the optimal conditioning strategy during early tissue formation may differ substantially from that required during later maturation stages. Consequently, stage-specific conditioning protocols may represent a promising strategy for promoting progressive tissue development. Stimulation intensity, frequency, duration, or modality could be adjusted according to the developmental status of the engineered tissue. Adaptive conditioning systems that continuously monitor tissue behavior and modify stimulation parameters accordingly may provide a more physiologically relevant approach to muscle maturation. The integration of sensing technologies capable of monitoring contractile force, stiffness, electrophysiological behavior, or metabolic activity, could support the development of such feedback-controlled maturation platforms (Chastagnier et al., 2026). Importantly, increasing the number of stimulation modalities does not necessarily result in superior maturation outcomes. Instead, the timing, intensity, sequence, and physiological relevance of applied cues likely play a greater role than the mere combination of mechanical, electrical, and perfusion stimuli. Future systems may evolve towards closed-loop conditioning platforms in which integrated sensing technologies, machine learning algorithms, and digital twin approaches continuously adapt stimulation protocols according to tissue performance and maturation status.

5.4 Construct-bioreactor interface mismatch

As architectural complexity increases, the integration of engineered tissues within bioreactor systems becomes increasingly challenging. While many current bioreactor studies utilize two-dimensional cultures or relatively simple three-dimensional models, the translation towards more physiologically relevant muscle tissues will require closer integration between construct architecture and bioreactor design.

This challenge extends beyond the selection of stimulation protocols alone. Complex engineered tissues frequently require dedicated anchoring systems, perfusion interfaces, stimulation electrodes, force-transducing elements, and integrated monitoring technologies to enable effective culture and functional assessment (Sprenger et al., 2026). Design considerations may therefore include scaffold dimensions, attachment strategies, stimulation directionality, perfusion access, and fabrication resolution. This is particularly relevant for anisotropic muscle tissues, where cellular orientation and force transmission pathways should ideally align with the applied mechanical or electrical stimulation.

Rather than designing scaffolds and bioreactors independently, future research should increasingly adopt co-design strategies in which both systems are optimized simultaneously. Such approaches may improve stimulation efficiency, enhance reproducibility, and facilitate integration of functional readouts such as contractile force measurements during culture.

5.5 Disconnected biofabrication metrics and functional maturation assessment

A final challenge arises from the discrepancy between the metrics commonly used to assess biofabricated constructs and those used to evaluate tissue maturation. Biofabrication studies frequently focus on parameters such as print fidelity, shape retention, cell viability, alignment, and protein expression. In contrast, bioreactor studies more commonly evaluate functional outcomes including contractile force generation, calcium handling, electrophysiological behavior, tissue mechanics, and long-term performance.

As a result, relationships between biofabrication parameters and functional maturation outcomes often remain unclear, making it difficult to compare findings across studies or identify the most critical determinants of tissue performance. Future work may therefore benefit from incorporating quantitative functional assessment throughout both fabrication and maturation stages. Establishing standardized evaluation criteria, normalization methods, and reporting practices may further facilitate cross-study comparisons and accelerate the development of clinically relevant muscle tissue engineering platforms (Shen et al., 2026).

6 Challenges and future outlook

Engineering functional skeletal, cardiac, and smooth muscle tissues requires the successful integration of biofabrication and tissue maturation strategies. As discussed throughout this review, major advances have been achieved in both areas. Biofabrication technologies now enable the generation of increasingly sophisticated multicellular and multimaterial constructs with controlled spatial organization, while bioreactor systems provide a wide range of mechanical, electrical, perfusion-based, and multimodal conditioning approaches capable of promoting tissue development. Despite these advances, engineered muscle tissues continue to exhibit substantial structural and functional differences compared to their native counterparts.

A central conclusion of this review is that structural biomimicry alone does not guarantee functional maturation. While advances in biofabrication have enabled increasingly faithful recreation of muscle architecture, tissue performance remains strongly dependent on the subsequent maturation environment. Likewise, the effectiveness of bioreactor conditioning is influenced by construct characteristics including biomaterial properties, cellular composition, tissue organization, and overall construct design. Consequently, future progress will require a shift away from the independent optimization of biofabrication and bioreactor technologies toward integrated construct-bioreactor development strategies.

Beyond technological integration, increasing biological complexity remains an important priority. Native muscle tissues consist of highly organized multicellular environments incorporating vascular, neural, stromal, and immune components that are still incompletely represented in most engineered models. Continued incorporation of human-derived cells, tissue interfaces, vascular networks, and supporting cell populations will be important for improving physiological relevance and translational potential.

Finally, the establishment of standardized functional assessment methods will be essential for accelerating progress within the field. While considerable advances have been made in both construct fabrication and maturation technologies, comparison between studies remains complicated by differences in stimulation protocols and outcome measures. The development of standardized benchmarks for evaluating muscle tissue maturation, such as specific force, may facilitate more direct comparisons between biofabrication and bioreactor studies and improve identification of factors that drive functional tissue development.

In conclusion, future advances in muscle tissue engineering will depend not only on the ability to fabricate increasingly complex tissues or develop more advanced bioreactor platforms, but on the successful integration of both technologies into unified workflows. Such approaches may help translate structural complexity into functional skeletal, cardiac, and smooth muscle tissues for applications in regenerative medicine, disease modelling, drug screening, cultured meat production, and biohybrid robotics.

Author contributions

JV: Investigation, Conceptualization, Writing – review and editing, Funding acquisition, Project administration, Writing – original draft, Validation, Visualization. MK: Project administration, Writing – original draft, Conceptualization, Supervision, Writing – review and editing. AS: Visualization, Writing – review and editing, Validation, Writing – original draft. SG: Writing – review and editing, Writing – original draft. DM: Writing – original draft, Writing – review and editing. SP: Writing – review and editing, Validation, Writing – original draft, Visualization, Supervision, Conceptualization. NP: Writing – original draft, Investigation, Visualization, Validation, Project administration, Methodology, Writing – review and editing, Conceptualization, Supervision.

Funding

The author(s) declared that financial support was received for this work and/or its publication. JV and NP would like to acknowledge the financial support of the Research Foundation Flanders (FWO) under the form of an FWO SB PhD fellowship (1SH7424N) and an FWO senior postdoctoral fellowship (12A1U27N), respectively. MK would like to acknowledge the financial support of the Interreg Flanders-Netherlands project B-SMARTT! (Project ID: Int6A035), co-funded by the European Union, European Regional Development Fund (ERDF), Interreg Belgium-The Netherlands (Vlaanderen-Nederland) programme and the Flemish Government through the Flemish Agency for Innovation and Entrepreneurship (VLAIO).

Conflict of interest

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The author DM declared that they were an editorial board member of Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

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