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REVIEW

Striated muscle function, regeneration, and repair

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Abstract As the only striated muscle tissues in the body, skeletal and cardiac muscle share numerous structural and functional characteristics, while exhibiting vastly different size and regenerative potential. Healthy skeletal muscle harbors a robust regenerative response that becomes inadequate after large muscle loss or in degenerative pathologies and aging. In contrast, the mammalian heart loses its regenerative capacity shortly after birth, leaving it susceptible to permanent damage by acute injury or chronic disease. In this review, we compare and contrast the physiology and regenerative potential of native skeletal and cardiac muscles, mechanisms underlying striated muscle dysfunction, and bioengineering strategies to treat muscle disorders. We focus on different sources for cellular therapy, biomaterials to augment the endogenous regenerative response, and progress in engineering and application of mature striated muscle tissues in vitro and in vivo. Finally, we discuss the challenges and perspectives in translating muscle bioengineering strategies to clinical practice.

Keywords Muscle · Cardiac · Skeletal · Tissue engineering · Stem cells · iPS

Abbreviations

CM	Cardiomyocyte
CSC	Cardiac stem cell
CV	Conduction velocity
CHD	Congenital heart defect
DGC	Dystrophin-associated glycoprotein complex
ECM	Extracellular matrix
FAP	Fibroadipogenic progenitors
hESC	Human embryonic stem cell
hiPSC	Human induced pluripotent stem cell
mESC-CM	Mouse embryonic stem cell-derived cardiomyocyte
MHC	Myosin heavy chain
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cells
NRVM	Neonatal rat ventricular myocyte
PIC	Pw1 interstitial cell
RyR	Ryanodine receptor
SR	Sarcoplasmic reticulum
SERCA	Sarcoplasmic reticulum Ca^{2+} ATPase
SC	Satellite cell
SIS	Small intestine submucosa
T-tubule	Transverse tubule

Introduction

The body possesses two types of striated muscle, cardiac and skeletal. Human skeletal muscles can be further categorized into slow-oxidative/Type I, fast-oxidative/Type IIa, and fast-glycolytic/Type IIb types based on their contractile and metabolic phenotypes [1]. Striated muscles are required for whole-body oxygen supply, metabolic balance, and locomotion. While structurally and functionally similar, the two striated muscles have vastly different sizes and regenerative capacities. In adult humans weighing ~70 kg, skeletal muscle tissue weighs ~27 kg comprising 35 % of the total body mass [2], while cardiac muscle is ~100

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times smaller weighing in average 270 g [3]. Skeletal muscle can regenerate in response to small muscle tears that occur during exercise or daily activity owing to the abundances of resident muscle stem cells called satellite cells (SCs), which upon injury activate, proliferate, and fuse to repair damaged or form new muscle fibers [4]. In contrast, cardiac muscle does not possess a cardiomyogenic stem cell pool and has little to no regenerative ability, with injury resulting in the formation of a fibrotic scar and, eventually, impaired pump function [5].

Functional deficit in skeletal muscle occurs with age, chronic degenerative diseases such as muscular dystrophy [6], defects in metabolism such as Pompe disease [7], and with large volume muscle loss due to trauma or surgical resection [8]. In these conditions, SC pool is often depleted due to the disruption of SC niche or continuous SC activation resulting in impaired muscle regeneration and chronic fibrosis [9]. Cardiac muscle dysfunction arises largely from narrowing of coronary arteries caused by vascular disease and less frequently from non-ischemic cardiomyopathy, congenital abnormalities, diastolic disease, and certain muscular dystrophies [10, 11].

In this review, we first discuss the structure, function, and regenerative potential of healthy striated muscles, representing the desired outcome of any cell, biomaterial, drug, or gene therapy for muscle disorders. We then review the progress made with cellular therapies, where immature cells are directly delivered in vivo, as well as cell-free therapies where biomaterials are implanted to augment and replicate the natural repair capacity of muscle tissue. Lastly, we review recent developments in engineering mature striated muscle tissues in vitro and highlight the hurdles that need to be overcome to translate these promising approaches to the clinic.

Striated muscle structure and function

Striated muscles are highly organized tissues (Fig. 1) that convert chemical energy to physical work. The primary function of striated muscles is to generate force and contract to support respiration, locomotion, and posture (skeletal muscle) and to pump blood throughout the body (cardiac muscle).

Ultrastructure

Under light microscopy, striated muscles have highly ordered ultrastructure consisting of sarcomeres, which are basic contractile units containing a central myosin-rich dark anisotropic (A) band and two actin-dominated light isotropic (I) bands [12, 13]. While the sarcomeric proteins are conserved among cardiac and slow-twitch and fast-twitch skeletal muscles, the existence of specific isoforms of these proteins contributes to observed differences in rates of muscle contraction and relaxation [14, 15]. All types of striated muscle contain a branched network of membrane invaginations called T-tubules that enable synchronous calcium release throughout the entire cell volume. The T-tubules contact the sarcoplasmic reticulum (SR) between the A and I bands in skeletal muscle and at the Z-disc in cardiac muscle. Dihydropyridine-sensitive voltage-gated Ca^{2+} channels in the T-tubules associate with the Ryanodine receptors (RyR) on the junctional face of the SR, converting action potentials to calcium release [16].

In skeletal muscle, the Ca^{2+} channel ($Ca_V1.1$) and RyR1 physically interact, producing a fast release of calcium from SR ($\sim$2 ms) without the need for extracellular calcium entry into the cell (Fig. 1c). In cardiac muscle, the Ca^{2+} channel ($Ca_V1.2$)-RyR2 complex requires extracellular calcium entry for the calcium release from SR, which significantly slows the maximal frequency at which cardiac muscle can contract and prevent generation of tetanus, a hallmark of skeletal muscle contraction [17] (Fig. 1d). Where the T-tubules and SR meet, the SR enlarges, fuses, and forms expanded chambers called terminal cisternae. In skeletal muscle the T-tubule meets with two terminal cisternae to form a triad, but only with a single terminal cisternae in cardiac muscle to form a diad. The triads enable sufficient supply of calcium from SR to sustain tetanic contractions.

Micro-/macroscopic structure

Despite possessing the same functional units, the microscopic structures of skeletal and cardiac muscle fibers are different. Individual skeletal muscle fibers arise from the fusion of many muscle cells, producing multi-nucleated linear fibers, millimeters to centimeters in length (Fig. 1a). In contrast, cardiac muscle consists of a cellular syncytium wherein individual cells are electromechanically interconnected in a branched pattern via specialized structures known as intercalated discs (Fig. 1b). Within the intercalated disc, gap junctions allow for a rapid propagation of electrical impulses (Fig. 1g), which results in a near-simultaneous depolarization of the entire cardiac syncytium. Unlike the fused skeletal muscle fibers, individual cardiomyocytes are much smaller ($\sim$120 μm in length) [20], contain centrally located nuclei, and remain predominantly ($\sim$65 %) mononucleated during all stages of human heart development [21], which is in contrast to a maturation-induced shift towards binucleation known to occur in murine hearts [22]. Finally, while skeletal muscle fibers are directly innervated by motor neurons, cardiomyocytes are excited via a conduction cascade that begins with

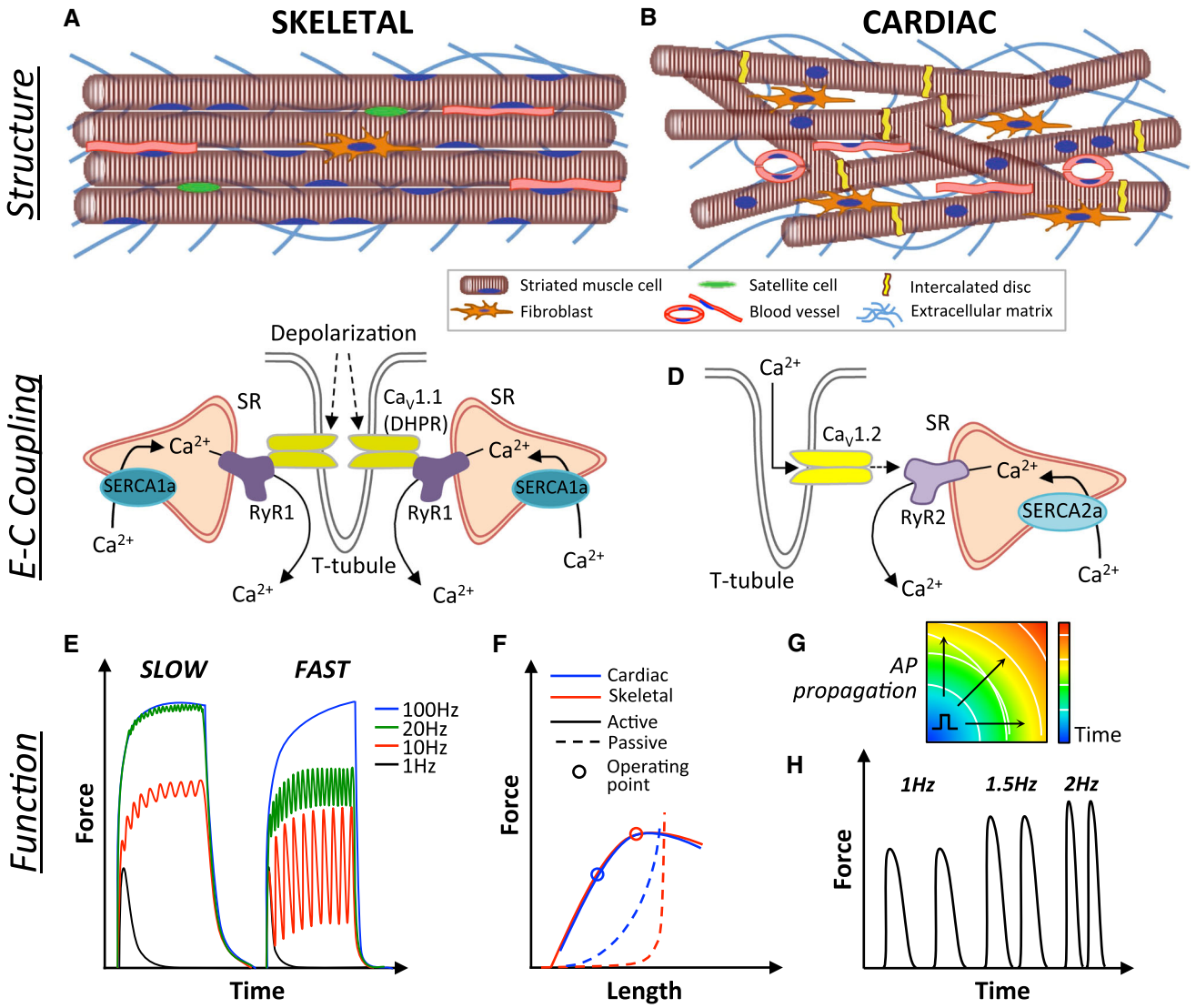

Fig. 1 Structure and function of striated muscles. **a** Adult skeletal muscle contains uniformly aligned, long multinucleated myofibers, blood vessels, and resident satellite cells, with fewer fibroblasts relative to cardiac muscle. **b** Adult cardiac muscle consists of a branched network of shorter cardiomyocytes connected via intercalated discs and surrounded by blood vessels and extracellular matrix secreted primarily by fibroblasts. **c** Skeletal muscle excitation–contraction (**e**–**c**) coupling begins with a depolarization-induced conformational change in L-type Ca^{2+} channels ($Ca_V1.1$, dihydropyridine receptor, DHPR) that triggers release of Ca^{2+} from two neighboring SR terminae via opening of the Ryanodine receptor (RyR1) channels, creating a triad (rather than a diad as in cardiac muscle) with the T-tubules. Calcium is pumped back into the SR via the SR-ATP-ase (SERCA1a). **d** Cardiac E-C coupling occurs through a Ca^{2+}-dependent Ca^{2+} release wherein T-tubular entry of extracellular Ca^{2+} through depolarization activated L-type Ca^{2+} channels ($Ca_V1.2$) triggers a release of Ca^{2+} stored in the sarcoplasmic reticulum (SR) via opening of the RyR2 channels. Calcium is pumped back into the SR via the SR-ATP-ase (SERCA2a). **e** Tetanic responses of slow and fast-twitch skeletal muscle fibers showing increased ability to recover from fast-paced stimulation in fast-twitch fibers. **f** Comparison of active and passive tension-length relationships in cardiac and skeletal muscle. Both striated muscles exhibit stronger active (contractile) force with increased muscle length followed by decay at higher levels of stretch (Frank-Starling relationship). While skeletal muscle operates close to the peak of its active force–length curve, cardiac muscle operates at the ascending limb of the curve to allow more forceful contraction at larger diastolic filling. Simultaneously, passive tension of cardiac muscle at its operating length is markedly higher than that of skeletal muscle, primarily due to higher stiffness of titin molecules within the sarcomeres. **g** Unlike skeletal muscle, cardiac muscle can propagate action potentials (APs) between myocytes that are connected via gap junctions. Schematic depicts an isochrone map showing AP propagation through cardiac muscle, from which conduction velocity can be measured. **h** Positive force-frequency relationship of cardiac muscle demonstrating increased force production at higher excitation rates

specialized pacemaking cells of the sinoatrial node and terminates at the ventricular cardiomyocytes.

Skeletal muscle fibers are encased in a basement membrane rich in collagen IV, heparin sulfate proteoglycans (HSPGs), and laminin, which plays a key role in force transmission to the outer three connective tissue layers, the endo-, peri-, and epimysium. These layers predominantly consist of Type I, II and III collagens synthesized by fibroblasts. Healthy skeletal muscle has a high volume ratio of muscle cells to fibroblasts, with fibroblast nuclei comprising 8–15 % of all nuclei in the tissue [23, 24]. In the heart, Collagen I is the main ECM protein made by cardiac fibroblasts that until recently were believed to be the most dominant cell type in the heart, with different abundances reported in different species [25–27]. Recent studies, however, identified endothelial cells as the predominant cell type in both mouse and human adult ventricles, representing 45–55 % of the total cell count (compared with ~30 % cardiomyocytes and ~15 % fibroblasts), although, similar to skeletal muscle, taking up only a small fraction of the ventricular volume [28]. While evidence for electrical coupling between fibroblasts and cardiomyocytes is present in vitro [29, 30], the ability of these cell types to form functional heterocellular gap junctions in vivo is still widely debated [31, 32]. Both in skeletal and cardiac muscles, blood is delivered to cells in a hierarchical manner with primary arteries progressively bunching into smaller vessels and capillaries [33, 34]. Greater capillary density is found in slow-oxidative than fast-glycolytic muscles [1, 35] and in epicardium compared to endocardium with an average of 1.3-2.5 capillaries being associated with each cardiomyocyte or myofiber [33, 36, 37].

Contractile function

Skeletal muscle force output is primarily achieved by summation and motor unit recruitment. Slow (10–30 Hz) and fast (50–100 Hz) fibers achieve peak force at different frequencies due to the speed of contraction and relaxation (Fig. 1e), which is dictated by the number of terminal cisternae and the fast or slow isoform dominance of sarcomeric and calcium handling proteins [1]. Although lacking a tetanic response characteristic of skeletal muscle, the human heart still displays a positive force-frequency relationship (Fig. 1h), largely attributed to an increased net uptake of Ca^{2+} into SR during faster heart rates [38]. Furthermore, force generation in skeletal muscle is typically controlled by motor unit recruitment, in order from smallest to largest, which results in an exponential increase in force and enables a single muscle to produce both delicate and explosive movements [39]. In contrast, cardiac muscle contracts as a syncytium and obeys an "all-or-none" phenomenon.

Both skeletal and cardiac muscles display a biphasic force–length relationship, which is, in part, explained by the sliding filament theory [40]. Lengthening of the muscle increases the overlap of myosin and actin filaments within each sarcomere, which produces a higher contractile force during the power stroke of the contraction cycle. Further increases in muscle length can decrease the myosin/actin overlap, causing a drop in the active tension (Fig. 1f). However, in cardiac muscle the predominant cellular basis for the force–length relationship is the increased affinity of troponin C to calcium, which yields opening of additional sites for binding of myosin to actin and permits greater force generation [41]. In contrast to skeletal muscle, which operates close to the peak of its active force–length curve, cardiac muscle operates on the ascending part of the curve to allow stronger pumping for increased ventricular filling (Fig. 1f). Furthermore, at their respective resting/working lengths, cardiac muscle experiences significantly higher passive tension than skeletal muscle (that has ~0 passive tension; Fig. 1f), reflecting the existence of much stiffer isoforms of titin and collagen in cardiac muscle [42].

Endogenous striated muscle repair

In response to injury, adult skeletal muscle exhibits robust regenerative response that involves a highly orchestrated action of multiple cell types. The most important cells are Pax7+ satellite cells (SCs), which are located between the sarcolemma and the basal lamina [43] and are essential for muscle regeneration [44]. During embryogenesis, myogenic transcription factors Myf5 and Mrf4 are transiently expressed to give rise to a significant number of SCs that persist throughout development [45, 46]. In adult muscle, SCs are a heterogeneous population with the majority of cells expressing Myf5 and being able to directly commit to myogenic differentiation [47]. SCs not expressing Myf5 preferentially self-renew and fill the SC niche but can divide asymmetrically to express Myf5 and support myogenesis [47, 48]. Regardless, the maintenance of SCs in adult muscle is dependent upon Myf5 highlighting the complex non-hierarchical regulatory network that controls SC stemness [49]. In response to injury and exercise, SCs that are typically quiescent become activated and contribute to muscle repair, as reviewed elsewhere [4, 50]. Briefly, upon activation, Pax7+ SCs begin to express MyoD, proliferate and divide asymmetrically to either: (1) commit to muscle differentiation by losing Pax7, and expressing Myf5, MyoD, and ultimately myogenin and then fusing into damaged fibers or forming *de novo* fibers

that express myogenin and Mrf4, or (2) maintain a quiescent state by losing MyoD expression and contributing to future rounds of muscle regeneration (Fig. 2a) [50, 51]. SCs comprise 2-7 % of total nuclear content of skeletal muscle, yet no equivalent cell type has been discovered in cardiac muscle. The c-Kit+ cell niche that emerged over the last decade is likely the closest cardiac relative of the SC niche, although the ability of c-Kit+ cells to generate functional cardiomyocytes has created a wide scientific debate that continues to this day [52, 53]. Regenerative process in skeletal muscle is controlled primarily by the innate immune response (Fig. 2a), with deletion of monocytes completely preventing muscle regeneration [54]. During the first stage of muscle repair, infiltrated neutrophils promote degeneration of muscle fibers while M1 macrophages stimulate a pro-inflammatory cytokine release and muscle cell lysis [55, 56]. Subsequently, the SCs undergo activation and differentiation, which is followed by ECM deposition, angiogenesis to revascularize regenerating tissue, and reinnervation of the new myofibers. Proliferating SCs also migrate bi-directionally along the longitudinal but not the horizontal axis of the degenerating fibers to aid in the redistribution of regenerating muscle progenitors [57]. Conversion to an M2 macrophage phenotype is critical for the repair process as it shifts SCs towards differentiation and away from proliferation seen predominantly with M1 macrophages [58]. Among the other cell types important for successful muscle regeneration, fibroadipogenic progenitors (FAPs) are known to promote myofiber formation through controlled ECM deposition and paracrine factors [59, 60].

In contrast to skeletal muscle, the adult mammalian heart lacks robust regenerative potential [5, 61]. However, shortly after birth mammalian hearts can still regenerate, as recently shown in the case of a newborn child with myocardial infarction [62]. In neonatal mice, this regenerative process is associated with the activation of epicardial-specific genes, angiogenesis, and a global proliferation of existing cardiomyocytes that restores cardiac function [63] and is dependent upon infiltration of neonatal macrophages [64]. In contrast, cardiac injury in adult heart produces a primarily fibrotic scar that leads to a decline in function. The cellular and molecular events responsible for this process can be broken down into early (<72 h) and late (>72 h) phases [65] (Fig. 2b). The early remodeling phase consists of neutrophil infiltration, activation of matrix metalloproteinasaes and early degradation of extracellular matrix (ECM), which leads to wall thinning and ventricular dilation. During the late phase, initial release of TGF-β1 from necrotic cardiomyocytes induces chemotaxis of fibroblasts and macrophages, proliferation and transformation into myofibroblasts, synthesis of Type I & III collagen, and eventual fibrosis. Pathological hypertrophy also develops as an adaptive response to the decreased contractile function and is largely initiated by the myofiber stretch following chamber dilation and neurohormonal activation, in particular via combined actions of norepinephrine, endothelin-1, and angiotensin II [65].

Striated muscle dysfunction

In contrast to certain amphibians [66] and fish [67], the endogenous repair mechanisms in mammalian skeletal muscle can only regenerate a finite amount of muscle tissue and can thus be overwhelmed by the deposition of fibrotic scar following volumetric muscle loss resulting from trauma or large resection [68]. Furthermore, in sarcopenia, skeletal muscle mass, function, and regenerative ability decrease with age and can cause or exacerbate health problems [69, 70]. Sarcopenia is characterized by a muscular atrophy, loss of muscle fibers, decreased number and size of motor units, and increased fibrosis and fat accumulation [71–74], and can likely be attributed to SC depletion and impaired SC function. In mice, aged satellite cells have reduced regenerative potential [75] due, in part, to defective FGFR1 signaling and consequent p38αβ MAPK activation which promotes SC exhaustion [76]. Inhibition of p38 MAPK restores the regenerative potential of aged SCs, and can restore force generation of muscles in aged mice to levels found in younger animals [75, 76].

Similar to skeletal muscle, impaired function of cardiac muscle can arise from various congenital or acquired diseases. In ischemic heart disease, the leading cause of morbidity and mortality in the world [77], the buildup of atherosclerotic plaques within coronary arteries increases with age and can cause tissue ischemia or necrosis (myocardial infarction) upon rupture, which can precipitate into congestive heart failure over time. Numerous myopathies, including autoimmune and viral myocarditis, can similarly lead to heart failure if left untreated. Diastolic cardiac dysfunction occurs due to pathological hypertrophy and stiffening of the heart walls that impairs chamber relaxation and filling during the cardiac cycle. The long-standing hypertension, ischemic injuries, aging, and aortic stenosis, as wells as certain metabolic diseases such as glycogen storage disease [78] can all cause diastolic dysfunction. Lastly, congenital heart defects (CHDs) are the most common type of birth defect and represent the leading cause of mortality in infants [79, 80]. Three of the top five common types of CHDs—ventricular septal defects, Tetralogy of Fallot, and atrial septal defects—all contain a defect in the heart wall that requires surgical correction when sufficiently large, and often multiple surgeries due to failed or inadequate grafts [81].

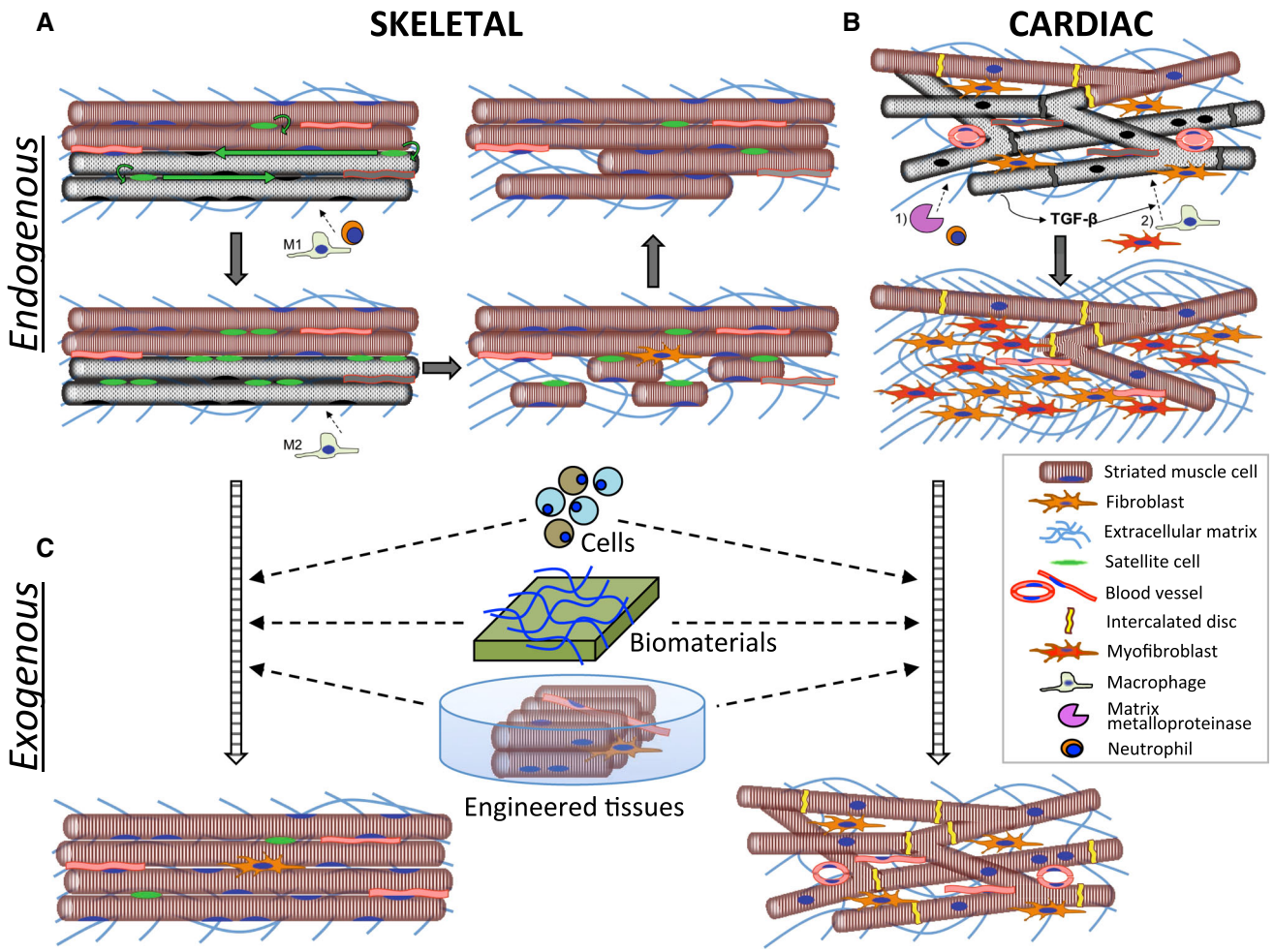

Fig. 2 Endogenous and exogenous repair of striated muscles. **a** Damage to skeletal muscle results in proliferation and migration of satellite cells (SCs) along the longitudinal axis of dying fibers (*gray*) and initial infiltration of pro-inflammatory M1-macrophages and neutrophils which aids in the degeneration of damaged fibers. Conversion to and infiltration of M2-macrophages stimulates SCs to differentiate and eventually fuse into functional myofibers. **b** Ischemic injury to cardiac muscle results in death of cardiomyocytes (CMs), an initial infiltration of neutrophils and upregulation of matrix metalloproteinases. Release of TGF-β from necrotic CMs induces late migration of macrophages and fibroblasts as well as transformation of fibroblasts into myofibroblasts, which secrete collagen to ultimately produce a fibrotic scar. **c** Striated muscle repair can be augmented via exogenous delivery of single cells, biomaterials with or without cells, as well as transplantation of in vitro engineered functional muscle tissues

Since a large number of proteins are expressed in all striated muscles, the same genetic defect can result in both cardiac and skeletal muscle disease. For example, mutations of proteins in the dystrophin-associated glycoprotein complex (DGC) or associated ECM proteins such as laminin and merosin can lead to a wide range of muscular dystrophies [82]. The DGCs link the sarcomeres to the ECM and are involved in the transmission of force and protection of the membrane from shear stress [83]. Thus, dystrophic muscles are damaged more easily, resulting in a repetitive degeneration/regeneration cycles, eventual exhaustion of the SC pool, muscle loss, and fibrosis [9, 84]. Still, not all skeletal muscles are affected equally by muscular dystrophy, with the ocular muscles being typically spared [85], but the diaphragm which is in constant use being the most severely affected muscle [86] and load-bearing muscles such as the gluteus maximus and the posterior muscles of the lower legs [87, 88] also being severely affected. Furthermore, different diseases can affect cardiac and skeletal muscles differently. Duchenne Muscular dystrophy (DMD), the most severe form of muscular dystrophy characterized by the complete loss of functional dystrophin, typically has a more severe skeletal muscle phenotype and a milder cardiac phenotype [89]. In contrast, Becker's Muscular Dystrophy (BMD), characterized by a partial loss of functional dystrophin, has a milder skeletal phenotype and a more pronounced cardiac phenotype, potentially due to BMD patients being more

physically active than DMD patients and placing more strain on their heart muscle [90, 91].

Therapies for striated muscle disorders

Cell injection therapies

The first cell-based approaches to improve function of injured or diseased striated muscles involved transplantation of stem or progenitor cells into the site of tissue damage. These strategies require expanding a suitable cell population in vitro to generate sufficient cell numbers for transplantation. The desired characteristics of the expanded cells would be to: (1) retain innate stemness or myogenicity during in vitro culture, (2) be immunoprivileged to obviate a need for long-term immunosuppression, (3) survive and robustly engraft upon transplantation, (4) Structurally and functionally integrate with host tissue, (5) repopulate and replenish the tissue-resident stem cell niches and (6) permanently improve muscle function following transplantation. Additionally, having cells that can be delivered throughout the circulatory system and efficiently home to damaged tissues would be desirable to avoid damage due to multiple intramuscular injections and when needed enable the uniform distribution of cells throughout the entire organ.

Skeletal muscle

Primary myoblasts and satellite cells Given the robust regenerative potential of satellite cells (SCs), it was hypothesized that expanding these cells in vitro and injecting them into damaged muscles may counter loss of muscle mass and function. Initial transplantations of in vitro expanded SCs into skeletal muscle; however, resulted in poor survival, motility, and engraftment/fusion with host myofibers as well as immunorejection [92]. The poor outcomes in these studies have been attributed in part to the in vitro conditions used to expand SCs [93, 94]. Traditional culture for SC expansion is optimized to maximize cell proliferation, which activates SCs and rapidly leads to their commitment and differentiation to myoblasts that can not be readily reverted back to a self-renewing state. Furthermore, committed myoblasts with high proliferation and differentiation potential undergo apoptosis following implantation of whole muscle cultures, with the cells that survive and engraft being the slow-dividing "stem-like" cells [95]. Selection and implantation of these slow-dividing cells promoted greater and longer-lasting muscle regeneration in vivo [96]. Recently, SCs cultured on 12 kPa compliant PEG hydrogels (mimicking stiffness of native skeletal muscle [97]) were shown to engraft more efficiently compared to cells grown on standard tissue culture dishes but still less efficiently than freshly isolated SCs [98], suggesting that mechanical cues of native SC microenvironment are an important but not the only determinant of SC "stemness" to be replicated during SC expansion in vitro.

Of note is that encouraging results have been recently reported in a Phase I/IIa clinical trial of myoblast transplantation in patients with oculopharyngeal muscular dystrophy, where the dystrophic phenotype is limited to pharyngeal muscles. Two years after autologous myoblast transplantation patients showed improved quality of life and no deterioration of swallowing function [99]. This suggests that despite low survival and engraftment, transplanting large numbers of myoblasts could still yield successful repair of small muscles. For larger muscle defects, improved culture conditions to increase the proportion of slow-dividing myogenic cells and/or self-renewing SCs will be required to develop efficient cell-based therapies. On the other hand, myoblasts have low engraftment efficiency when delivered systemically, which prevents their use in treatment of congenital diseases that affect all muscles in the body, thus necessitating an alternative cell source.

Human pluripotent stem cell-derived skeletal muscle cells Human pluripotent stem cells (hPSCs) have the potential to overcome the issue of in vitro satellite cell expansion due to their unlimited proliferative potential. The generation of muscle progenitors from hPSCs has been achieved by utilizing cell sorting for defined extracellular markers, direct genetic reprogramming, or small molecule inhibitors. After the first published protocol for derivation of muscle progenitors from hESCs by sorting for $CD73^{+}$/$CD56^{+}$ cells [100] that has been difficult to reproduce, researchers have overexpressed the myogenic transcription factors Pax7 [101–103] or MyoD [104–106] in hPSCs to generate muscle progenitors in as little as 5 days. These cells efficiently differentiated to myotubes in vitro, fused more efficiently to existing myofibers in vivo than human myoblasts and improved muscle function. Still the risk of undesired genetic recombination or reactivation makes this approach unlikely to be used clinically. Recently small molecules have been used to differentiate hPSCs to muscle progenitors capable of fusing in vitro and with mouse myofibers in vivo [107–110] by protocols that combined GSK3β inhibition and IGF-1, HGF, and/or FGF2 supplementation, with overall efficiency that could be increased via cell sorting [108] or the addition of forskolin [109].

Interstitial cells, fibroadipogenic progenitor cells, pericytes and mesoangioblasts In addition to SCs, other tissue-resident cells with regenerative potential have been recently identified in rodent and human skeletal muscle. Specifically, interstitial muscle progenitor cells are a heterogeneous cell pool that does not reside under the basal lamina. A fraction of these cells termed Pw1 interstitial cells (PICs) express Pw1/Peg3, an early marker of the myogenic lineage [111], and do not express Pax7 [112]. Developmentally, PICs have distinct origin from SCs but can give rise to SCs indicating that they are upstream of SCs in muscle precursor lineage hierarchy. PICs may also contribute to muscle regeneration through paracrine release of pro-myogenic and differentiation factors. Fibroadipogenic progenitors, which are a subset of PICs, are activated in response to muscle injury and shown to promote murine muscle regeneration via paracrine growth factor release [59]. They are also identified in human skeletal muscle [113]. However, in response to chronic injury or aging, they can contribute to fibrosis and fat accumulation [114, 115]. Further delineation of how exogenous transplantation of PICs regulates muscle regeneration is required but given their greater abundance in extraocular muscles, which are unaffected by sarcopenia and muscular dystrophy [116], PICs are a promising cell source for muscle regeneration.

Other types of PICs include pericytes, which surround endothelial cells in microvessels, and mesoangioblasts, which are thought to be a subset of pericytes or pericyte precursors that exhibit multi-lineage developmental potential [117–122]. Pericytes display greater spontaneous myogenic differentiation in vitro than mesoangioblasts but in contrast to SCs, do not express myogenic regulatory factors until after myotube formation [121]. Pericytes can be further classified into type 1 and type 2 [120]. Type 1 pericytes express the adipogenic marker PDGFRα and contribute to fat accumulation and do not appear to be directly involved in muscle regeneration. They have significant overlap with FAPs in terms of cell marker expression and cell behavior in vivo and in vitro and therefore under certain circumstances may secrete factors that enhance muscle self-repair. Conversely, type 2 pericytes do not express PDGFRα, are highly myogenic both in vitro *and* in vivo and upon implantation fuse into damaged myofibers. When delivered intra-arterially, pericytes and mesoangioblasts show higher survival, retention and fusion into myofibers compared to satellite cells and distribute throughout all muscles in the body [123]. Furthermore, these cells proliferate extensively in vitro and can be genetically modified making them an attractive cell source for treatment of congenital myopathies such as muscular dystrophy [124–126]. Additionally, iPSCs derived from mesoangioblasts fuse more efficiently with skeletal muscle compared to iPSCs derived from fibroblasts suggesting that the mesoangioblasts may be a more ideal source from which to generate iPSC-derived myogenic progenitors [127]. To date, all published work with mesoangioblasts and pericytes has been preclinical, being performed in mice and dogs, and it is currently unclear if this promising cell therapy approach will translate to humans and be clinically feasible. Interestingly, PW1 is required for mesoangioblasts to cross blood vessel walls and engraft into myofibers and silencing of PW1 inhibits myogenic differentiation potential of mesoangioblasts [128]. Therefore, engineering and/or selecting cells with high PW1 expression may enhance the efficacy of cell transplantation but this has yet to be tested.

Cardiac muscle

Owing to the vast prevalence of heart disease throughout the world, a wide variety of cell types have been used as cardiac cell therapies. Recent reviews have nicely summarized the most important clinical trials using autologous cell types [129, 130], which can be broadly grouped into three main categories: (1) skeletal myoblasts, (2) adipose- and bone marrow-derived cells, and (3) Resident cardiac progenitors and cardiosphere-derived cells.

Myoblasts Given the limited success of myoblast transplantation into diseased skeletal muscles and the similarities of the two types of striated muscle, early investigations aimed to determine whether myoblasts could also be used for cardiac repair. Initial small non-randomized phase I trials showed a functional benefit following myoblast transplantation but the interpretation of these results is difficult due to the use of confounding treatments such as left ventricular assist device or coronary bypass grafting surgery [131–133]. However, larger Phase II trials found no improvement in LVEF [134, 135] and lack of electromechanical integration between donor myotubes and host myocardium, which even if possible could increase the incidence of arrhythmias [136]. The larger Phase II trials did find an attenuation of LV remodeling and decrease in LV volume, which was attributed to myoblast paracrine action [137, 138]. Overall, the use of myoblasts for cardiac myoplasty is likely to never permit sufficient return of function that would outweigh the incidence and risks of arrhythmia, and thus alternative cell sources have been pursued.

Bone marrow- and adipose-derived cells Bone marrow-derived cells have been a natural source for cell therapy owing to their relative ease of isolation and the presence of stem cells that have the ability to differentiate into various tissues. These cells are a mixed population of largely

undifferentiated cells that consist of early committed cells, hematopoietic and endothelial progenitor cells (~2–4 %), as well as mesenchymal stem cells (MSCs, <0.1 %) [139]. MSCs are a unique multipotent cell group of mesodermal origin characterized by expression of the surface antigens CD73, CD90, CD105, CD44, CD106, and CD166, but not CD34, CD31, CD14, CD45, CD133, or CD105 [140]. Given the relative scarcity of these cells in the bone marrow, MSCs have also been successfully isolated from numerous other organs, including periosteum, synovial membrane, skeletal muscle, skin, pericytes, peripheral blood, deciduous teeth, periodontal ligament, umbilical cord, and adipose tissue, the latter of which holds much promise given the ubiquitous presence of adipose tissues in the body and limited patient morbidity and discomfort upon isolation [141]. To date, two recent meta-analysis studies assessed a combined 82 clinical trials (close to 5000 patients enrolled) using bone marrow-derived cells for cardiac repair and found modest yet significant improvements in LVEF and reduction in scar size (both ~4 %) [142, 143]. However, a 3rd meta-analysis comprising 22 clinical trials failed to identify a significant effect of bone marrow cell treatment on MRI-derived cardiac parameters or clinical outcomes [144]. As typical with cellular therapies, this discrepancy likely stems from differences in the exact cell type, amount of injected cells, route of delivery, and timing and number of injections, suggesting that even larger studies may be needed to establish the utility of bone marrow-derived cells for treatment of heart disease. Similarly, a recent power meta-analysis involving 1225 patients found a statistically significant improvement in LVEF following MSC treatment, with a majority of clinical trials that demonstrated decreased LV dilation and remodeling employing exogenous MSCs [145]. Further trials are ongoing or planned to determine the exact mechanisms of functional improvements and identify MSC subfractions (such as CD133+ cells [146]) with enhanced therapeutic benefits.

Resident cardiac progenitors and cardiosphere-derived cells Since the ground-breaking discovery of endogenous cardiac progenitors ("cardiac stem cells", CSCs) over a decade ago [147], numerous pre-clinical studies have assessed whether in vitro expansion followed by administration of these cells into the heart can be a viable therapeutic approach for cardiac repair. CSCs are an exceptionally rare c-Kit$^+$/Lin$^-$ population, comprising 0.002–0.005 % of all cells in the heart, and are typically subcultured from cardiac biopsies [130]. Alternatively, cell clusters termed cardiospheres can be cultured from endocardial biopsies and dissociated to obtain cardiosphere-derived cells, a heterogeneous population predominantly containing c-Kit$^+$/Lin$^-$ CSCs and CD105$^+$/CD90$^+$/CD45$^-$ non-hematopoietic cardiac mesenchymal cells [148]. Recent advancements in endocardial biopsy techniques have significantly improved the ability to isolate CSCs and cardiosphere-derived cells [149]. A recent randomized controlled phase I trial (SCIPIO) demonstrated that intracoronary administration of purified c-Kit+ CSCs significantly improved LV systolic function (up to 12 % increase) and reduced infarct size after 1 year when administered to patients suffering from chronic ischemic cardiomyopathy [150]. Another randomized controlled phase I clinical trial (CADUCEUS) similarly showed that administration of cardiosphere-derived cells (95 % CD105$^+$ without c-Kit$^+$ cell purification) improved regional function of infarcted myocardium and decreased scar size, this time in patients with a more acute LV dysfunction after a myocardial infarction [151]. As such, these studies have paved the way for future phase II clinical trials, which will aim to establish the curative effect of cardiosphere-derived cells and/or CSCs in both acute and chronic settings of ischemic cardiomyopathy.

Human pluripotent stem cell-derived cardiomyocytes Since the first successful cardiac differentiation of human embryonic stem cells (hESCs, [152]) and induced pluripotent stem cells (hiPSCs, [153]), hPSC-derived cardiomyocytes (hPSC-CMs) have rapidly gained interest for use in heart repair by being the only currently available source of functional human cardiomyocytes. To date, hPSC-CMs have been only tested in infarcted hearts of mice [154], rats [155–158], guinea pigs [159, 160], pigs [161], and most recently, Macaque monkeys [162], but not in humans. A majority of these studies reported some structural and functional improvements (decreased scar size, thicker heart wall, increased LVEF, etc.; for a recent review see [163]), although the Macaque studies have also risen concerns about the potential arrhythmogenicity of the therapy. Despite this limitation, the cryopreservation techniques developed by the Murry's group that allowed them to transplant 1 billion cardiomyocytes into the non-human primate hearts [162] is an important milestone prior to clinical translation, and it is foreseeable that in the near future hPSC-CM therapies will progress to clinical trials.

Cell-free, biomaterial therapies

An alternative to cell transplantation is the injection of biomaterials that stimulate different aspects of endogenous repair process or provide favorable mechanical environment to enhance survival of muscle cells. This approach decreases the costs and circumvents technical issues associated with expanding large numbers of patient specific cells, thus permitting the treatment to start sooner after injury. Additionally, the ability to deliver biomaterials in a

liquid form has potential for repairing injuries of any shape or size.

Skeletal muscle

Promising biomaterials for repair of large skeletal muscle defects are decellularized scaffolds, where native tissue is stripped of cellular material to prevent a host immune response. When implanted, decellularized scaffolds show a progression of events that mimic natural tissue repair [164]. In practice, decellularization protocols are intended to provide a balance between sufficient cellular removal and maintenance of ECM structure. Cellular debris in decellularized scaffolds [165, 166] and excessive modification of native ECM structure with cross-linkers [167] are both associated with poor remodeling outcomes. Despite the clinical use of decellularized scaffolds, the Food and Drug Administration has not established standards for tissue decellularization, resulting in high variability between different manufacturers and variable functional outcomes upon implantation [168].

The gastrointestinal small intestine submucosa (SIS) and bladder are the most commonly used tissues to derive acellular scaffolds for skeletal muscle repair, with SIS showing the same level of muscle regeneration compared to a skeletal muscle derived decellularized ECM [169]. In animal models, acellular scaffolds can restore muscle function though the level of functional repair can vary significantly [164, 170, 171]. Preliminary work in humans has mirrored the findings of animal studies, namely a variable functional recovery following scaffold implantation [164, 172]. It should be noted, however, that in the human studies scaffold implantation occurred months to years after volumetric muscle loss injury, which could preclude more favorable outcomes expected to result from earlier implantations. In addition to technical improvements in acellular scaffolds, combined implantation of cells and scaffolds [171] as well as optimization of physical therapy may further improve functional recovery in these patients [173].

Cardiac muscle

In terms of applicability in the heart, the SIS-ECM decellularized matrix has been demonstrated to be feasible and safe in 40 patients with congenital heart disease [174], while its utility for the repair of ischemic myocardium has been shown in murine [175, 176] and rabbit [177] models of myocardial infarction with moderate improvements in cardiac function. Similar to the SIS-ECM, decellularized matrix from rat [178] or porcine [179, 180] hearts has been used for myocardial repair in rat [178, 179] as well as porcine [180] models of myocardial infarction, the latter being delivered via a percutaneous transendocardial injection, demonstrating potential translatability to human therapies. Outside of decellularized matrices, a variety of scaffold materials have shown promising results in animal models of MI, including natural derivatives such as fibrin, alginate, collagen, chitosan, Matrigel, hyaluronic acid, and Gelfoam/gelatin, as well as synthetic materials such as self-assembling peptides and polymer-based systems (reviewed in detail in [181, 182]). In these studies, biomaterial injections in peri-infarct zone mainly served to reduce the compliance mismatch between the scar and remote myocardium and consequently promote cell survival and favorable remodeling of the heart. Additionally, numerous studies have demonstrated further therapeutic benefits by incorporation of growth factors (bFGF, TGF-β1, IGF-1, VEGF, Neuregulin-1β) and/or cells in the injected biomaterials [183–185]. Many of such studies continue to be performed in small animals; however, studies in larger animal models [186] are required to develop effective treatment strategies with prospect for clinical translation.

Engineered tissue therapies

The inability to engraft sufficient viable cells after transplantation by a bolus injection [187] has prompted development of alternative approaches to pre-engineer a striated muscle tissue in vitro followed by its implantation at the injury site in vivo. In an ideal case, the advantages of this approach are that tissue function is known prior to implantation, tissue implants can be preconditioned for maximized survival and function, patients can expect a more immediate return of function, restoration of large defects can be performed with a single intervention, and any adverse immune response is localized. It is accepted, although debatable, that the key to engineering a functional tissue in vitro is to best replicate the in vivo milieu, which can be achieved by providing cells with an appropriate ECM, biochemical, and mechanical microenvironment to support maximal differentiation and maturation. The ECM should be biocompatible and biodegradable, provide a high surface area for cell adhesion and structural support, and amplify autocrine and paracrine effects by minimizing diffusion distances [188, 189]. Furthermore, cells are exquisitely sensitive to the stiffness to which they are exposed to [190, 191] requiring that the provided ECM should be of similar stiffness to that of native tissue in vivo. The similar ECM protein composition and stiffness of skeletal (12 kPa) [97, 98, 191] and cardiac (8-18 kPa) [192, 193] muscles have resulted in the use of similar biomaterials for their in vitro tissue engineering.

Skeletal muscle

The most abundant ECM protein in adult striated muscles is collagen type I [194], and thus the first engineered skeletal muscle was produced by embedding avian myotubes within a collagen I hydrogel anchored between two fixed points [195]. This was followed by demonstrations that murine C2C12 myoblast cell line can undergo myogenesis (i.e., proliferate, fuse, and differentiate) within a 3D hydrogel environment [196–199]. Mechanical tension between the anchor points within hydrogel promoted myoblast alignment and stimulated muscle growth. Compared to culture in traditional 2D dishes, the 3D environment permitted longer culture times and greater maturation levels. This method has been used to engineer muscle from primary rodent [200–204] myoblasts but active forces were only generated when collagen was mixed with matrigel. The only reported functional engineered muscle using a pure collagen matrix was constructed by alternate layering of rat myotubes and collagen [205] and yielded specific forces of 2 mN/mm^2, significantly smaller than 230 mN/mm^2 measured in native muscle [206].

More recent studies have utilized fibrin hydrogels that permit significant matrix remodeling and ECM synthesis, and exhibit stiffness resembling that of skeletal muscle [207, 208]. The first fibrin-based engineered skeletal muscle constructs utilized primary neonatal rat myoblasts and generated specific forces (36 mN/mm^2) ~6 times lower than native adult rat muscles but greater than collagen-based muscle constructs [209]. Further improvements in specific force to approach values of 100 mN/mm^2 [210] have been achieved by including Matrigel [211], a matrix containing laminin and collagen IV proteins found in the basal lamina, dynamic culture and decreased tissue size to maximize nutrient and oxygen delivery [211, 212], and using a highly fusigenic progenitor population [212]. Overall, the absolute and specific forces generated by engineered muscles made of neonatal rat myoblasts have been far higher than those reported when using cells from other species [213–215], including human myoblasts which under similar conditions produced specific forces of 7.2 mN/mm^2 [215].

An alternative approach to use of hydrogels/scaffolds to support muscle formation is to allow cells to secrete their own ECM and self-organize into a 3D tissue. Using saran wrap as a cell culture substrate and adding fibroblasts to secrete sufficient ECM enabled the self-assembly of muscle tissue constructs that expressed adult isoforms of myosin heavy chain [216]. This method was improved by replacing saran wrap with laminin, the major component of the basal lamina [217]. While rat skeletal muscle cells in these tissues generated a specific force of 4.1 mN/mm^2, primary mouse cells generated 15.6 mN/mm^2, possibly due to the greater ECM secretion by fibroblasts to support muscle differentiation and force transmission. The long time to formation (~35 days) and challenges with scale-up have limited utilization of this technique compared to use of natural or synthetic scaffolds. Alternatively, self-assembly of large-area muscle constructs has been achieved using a cell sheet engineering technique in which mixtures of muscle cells and fibroblasts were cultured on dishes coated with thermoresponsive polymer poly(*N*-isopropylacrylamide) until they generated sufficient ECM and then detached from tissue culture plates by decreasing temperature [218–220]. Stacking of the sheets can allow generation of thicker muscle tissues and incorporation of endothelial and neuronal layers [218, 219]; however, functionality of these muscle constructs has yet to be reported.

While state-of-the-art in vitro engineered skeletal muscle tissues can display postnatal levels of generated contractile force and physiological length-tension and force-frequency relationships [210], their twitch:tetanus ratios mostly resemble embryonic state, although this may be dependent upon the geographical origin of the culture serum [221]. Furthermore, engineered muscles retain the contractile and metabolic phenotype specific to a muscle used for cell isolation [213, 222] and have been recently designed to successfully regenerate in vitro in response to cardiotoxin injury [210], thus exhibiting features characteristics of native mature muscle. Still, the myofiber diameter and functional parameters of engineered muscle remain inferior to those typically found in adult skeletal muscles. Generation of more complex tissue constructs containing additional non-muscle cell types may yield improved properties and utility of engineered muscles. For example, the addition of fibroblasts [223] or their paracrine factors [224] enhanced engineered muscle formation, though excessive fibroblasts can negatively impact force generation. Muscle–tendon units have been engineered by fabricating composite scaffolds to simultaneously form muscle and tendon *de novo* [225, 226], or by using native tendons [227, 228] or bone-tendon structures [229] as engineered muscle anchors. Addition of motor neurons [230–232] or spinal cord explants [233] resulted in the formation of neuromuscular junctions and promoted force generation of engineered muscle [233]. Whilst engineering a muscle–tendon-bone unit is expected to benefit in vivo implantation, it is unclear if neuron-muscle implants would integrate into the host neuronal system. Alternatively, factors that promote acetylcholine receptor formation and clustering on muscle fiber sarcolemma, such as agrin, may provide more benefit to functional integration of engineered muscle implants in vivo [234–236].

Although soluble factors and co-culturing with other muscle-resident cells are likely to promote myofiber maturation, engineering an adult-like muscle construct in vitro will require additional biophysical and metabolic cues. The continuous passive stretch applied to skeletal muscle by bone growth is critical for muscle development [237] while cyclic mechanical loading is crucial for regulating adult muscle mass [238]. Replicating these distinct loading regimes with ramp or cyclic stretch resulted in improved myotube alignment, hypertrophy, and mass of engineered muscle [239–242]. In addition to mechanical loading, tonic and voluntary muscle contractions induced by functional innervation are required for complete muscle development [243, 244], maintenance of muscle mass [245], and regulation of muscle fiber phenotype [246]. Thus application of electrical stimulation to mimic neuronal input induced rapid increase in force generation [222, 247–251], while mimicking fast and slow neural activation patterns directed engineered muscle contractile and metabolic phenotype and protein expression towards particular fiber type [213, 222, 248]. While the above methodologies will support maturation of engineered muscle in vitro and survival and functional integration in vivo, the scale-up of tissue size along with sufficient vascular supply remain as major roadblocks to clinical translation.

Cardiac muscle

Almost a decade after the emergence of the first tissue-engineered skeletal muscles, embryonic chick cardiomyocytes were embedded in a collagen gel loaded between Velcro-coated glass tubes to create the first tissue-engineered cardiac muscle [252]. These early cardiac tissues generated contractile stresses (specific forces) of 0.2–0.3 mN/mm^2 and showed length- and Ca^{2+}-dependent force increase characteristic of native myocardium. Owing to their relative ease of isolation and low cost, neonatal rat ventricular myocytes (NRVMs) have been the first, widely utilized mammalian cell source for cardiac tissue engineering. The first NRVM-based cardiac tissues were engineered by Bursac et al. in 1999 using fibrous polyglycolic scaffolds [253], followed by the first gelatin mesh-based cardiac tissues implanted by Li et al. [254]. Since then, NRVMs were utilized in 3D alginate scaffolds [255], collagen gels [256, 257] and collagen/Matrigel matrices [258], and have progressed towards the use in scaffold-free cell sheets [259] and cylinders [260], fibrin gels [261–263], and fibrin/Matrigel constructs [264, 265], the latter of which have generated contractile stresses of ~ 9 mN/mm^2 [264], still significantly lower than those measured in adult rat myocardium (~ 70 mN/mm^2 [266, 267]).

In addition to force of contraction, which is arguably the most critical functional output of engineered skeletal muscle, the engineered cardiac muscle must also replicate the fast action potential propagation (assessed by measuring conduction velocity, CV) that allows the native heart to rapidly contract in an orderly fashion. In terms of cardiac repair applications, mature electrophysiological properties of engineered cardiac tissues, including high conduction velocity, are necessary to ensure electrical safety of the therapy, while high contractile forces would contribute the therapeutic efficacy. To this end, several studies with NRVMs [264, 268–272] have utilized the technique of optical mapping, whereby incubation of engineered tissues in a voltage-sensitive dye such as Di-4-ANEPPS and stimulation by a point electrode allow CV measurement by analyzing the spatial spread of action potential wavefronts. Measured CVs have ranged from 14.4 cm/s [268] to 36.1 cm/s [272], still inferior to values reported for adult rat ventricular tissues (66–69 cm/s [273, 274]). Beside the evaluation of the basic tissue-scale functional parameters, robust formation of T-tubules (hallmark of excitation–contraction coupling maturation) was reported in NRVMs after 3–4 weeks of culture in fibrin-based constructs [272]. While these results were obtained without any external stimulation, application of chronic cyclic stretch to better mimic the in vivo cardiac mechanics has also yielded the T-tubular formation in NRVMs [258]. Recently, chronic electrical stimulation (4 Hz over 5 days) yielded a positive force-frequency relationship, which despite being at sub-physiological rates and small in magnitude, is still the first demonstration of an important adult-like physiological response [275].

With the advent of stem cell research, experiences with engineering of NRVM constructs have been applied to generate cardiac tissues from mouse [276–280] and, more recently, human embryonic and induced pluripotent stem cell-derived cardiomyocytes, ESC-CMs and iPSC-CMs [281]. In particular, numerous groups have engineered 3D cardiac tissues from hESCs and hiPSCs [163, 282–285] that generated specific forces ranging from 0.08 mN/mm^2 [286] to 11.8 mN/mm^2 [287] and conduction velocities ranging from 4.9 cm/s [288] to 25.1 cm/s [287, 289]. Thus, further progress in the field will be necessary to bring the state of human cardiac tissue engineering closer to replicating the robust phenotype of the adult human myocardium (specific forces of 25-44 mN/mm^2 [290, 291], average conduction velocity of ~ 50 cm/s [292]). In particular, as hESC- and hiPSC-CMs are structurally and functionally more similar to human fetal than neonatal or adult CMs [293], numerous strategies have been attempted to improve maturation of cardiomyocytes, including long-term culture, electrical and mechanical stimulation, supplementation with growth factors and hormones such as IGF-1 and T3, and fostering heterocellular interactions with non-myocytes [294–296]. For example, 3-4 month 2D

culture of hPSC-CMs has produced structurally and electrophysiologically more mature cardiomyocytes [297], while 1-year 2D-cultured hPSC-CMs demonstrated appearance of M-bands (in <10 % of CMs), a hallmark of advanced sarcomeric maturation [298]. Recently, a 4-month long culture within 3D PEG (poly(ethylene glycol))-fibrinogen hydrogels yielded the formation of T-tubules in hPSC-CMs [282]; still the CM volume in all these studies remained significantly smaller than that of the adult CMs.

Since long-term cultures are obviously time and resource intensive, much recent focus has been placed on accelerated maturation of human cardiomyocytes in vitro. Application of chronic electrical stimulation to engineered tissues improved hPSC-CM ultrastructural organization, contractile force generation, electrophysiological properties, and conduction velocity [299, 300], while cyclic stretch improved hPSC-CM alignment and hypertrophy [286]. Treatment of hPSC-derived cardiac tissues with thyroid hormone T3, which normally stimulates cardiac maturation during development, has led to increased cardiomyocyte size, sarcomere length, and contractile force, all indicative of structural and functional maturation [295]. Furthermore, several groups have demonstrated the crucial role that non-myocytes, primarily fibroblasts, smooth muscle cells, and endothelial cells, play in the development of functional 3D cardiac tissues [276, 286–289]. However, there exists a delicate balance in the cardiomyocyte to non-myocyte ratio, as insufficient non-myocytes will inadequately remodel and compact a 3D tissue, while their overabundance can impede the formation of a functional, electromechanically coupled syncytium [253]. Studies of various cell ratios using mESC-derived CMs suggest that as few as 3 % fibroblasts are enough to remodel a tissue matrix without impeding inter-myocyte connections [276], while a moderately high hESC-derived cardiomyocyte fraction (60–80 %) is optimal for generating human 3D tissues with highest forces of contraction [287]. Overall, success of the above individual modifications has been modest, and it is likely that achieving a true, adult cardiomyocyte phenotype in vitro will entail more sophisticated and mechanistically driven approaches to provide the key time-varying environmental cues present in the postnatal cardiac development (e.g. biomechanical, metabolic, endocrine, cell–matrix, cell–cell, etc.).

Engineered tissue vascularization and implantation

While the above strategies are expected to result in successful generation of mature striated muscles suitable for disease modeling and drug development in vitro, one of the main obstacles to successful therapies in vivo will be the ability to rapidly supply implanted muscle cells with oxygen and nutrients from the host circulation. The high metabolic demand of any larger size avascular striated muscle implants would result in rapid consumption of diffused oxygen within the tens of microns from the host capillaries leading to hypoxia and cell death. At least in the case of skeletal muscle implants, some regeneration could occur from resident satellite cells [210, 301] while similar effects in cardiac tissue constructs would only be expected if implanted cardiomyocytes remained proliferative in vivo. Formation of pre-vascularized tissues in vitro prior to implantation and/or conditions to accelerate vascularization in vivo offers avenues to improve implant survival and enhance therapeutic effects.

Skeletal muscle

In early studies, pre-vascularized engineered skeletal muscle tissues formed by tri-culture of myoblasts, endothelial cells (ECs) and fibroblasts in scaffolds composed of 50 % poly-(L-lactic acid) (PLLA) and 50 % polylactic-*co*-glycolic acid (PLGA) [302] showed improved cell survival and vascularization compared to tissues formed by co-culture of myoblasts and endothelial cells. In these constructs, fibroblasts were necessary to stabilize and increase formation of lumen structures, while increased tri-culture time prior to implantation further enhanced survival and function of muscle implants in vivo [303]. On the other hand, acellular bladder matrix, which naturally supports rapid angiogenesis and vascular infiltration [304], allowed the use of only ECs and muscle progenitor cells yielding vascularization, neuron infiltration, and maturation of engineered muscle tissue in vivo [305]. As skeletal muscle requires an axial vascular pedicle to maintain function and develop adequate vascularization [306], an arteriovenous (AV) loop connecting an artery and vein was used to provide such a pedicle [307–309], and combined with a tri-culture scaffold in vivo allowed the repair of large muscle defects [310]. Alternatively, engineered muscle tissues were placed along the femoral vessels to provide an axial vascular supply leading to the formation of viable myofibers throughout the tissue cross-section [306, 311]. These studies, among others, are highlighted in Table 1, which summarizes the function, vascularization, and in vivo tissue repair of in vitro engineered skeletal muscle implanted into various animal models.

Other approaches have involved incorporation of vascular endothelial growth factor (VEGF) into biodegradable polymer scaffolds or engineering of myoblasts to secrete angiogenic factors [312–314]. The sustained levels of VEGF by these methods result in far greater angiogenesis than bolus VEGF delivery. The results of these studies can be further improved by co-delivery of pro-myogenic

Table 1 Summary of in vivo skeletal muscle repair and vascularization via implantation of in vitro engineered tissues

Cell source	Cell types	Scaffold	Model	In vitro function	In vivo function	Vascularization	Effects	References
Rat	Neonatal MPCs	Fibrin/ Matrigel	Dorsal skin-fold window chamber	47.9 ± 4.1 mN/mm^2	65.7 ± 8.9 mN/mm^2	Host-derived vasculature. Implant highly vascularized and perfused	Continued myogenesis, vascularization, and increase in force generation during 2 weeks implantation period	[210]
	3wk rat MPCs	Bladder acellular matrix	TA volumetric muscle loss	N/A	Yes	Not reported	Positive responders show ~60 % functional improvement to non-treated VML injury. Negative responders show no improvement	[171]
	MPCs and MSCs	Laminin	TA volumetric muscle loss	121 ± 9.6 μN	Yes	Host-derived vasculature. Robust capillary network	Myotendinous junctions show greater maturation. Improved TA force but not to level of control	[341]
	Adult soleus MPCs	Fibrin	Femoral vascular pedicle	N/A	35.4 ± 62.2 mN/mm^2	Host-derived vasculature	3 weeks post-implantation, muscle shows mature sarcomeres	[306]
	Adult soleus MPCs	Fibrin	Femoral vascular pedicle	N/A	Yes	Host-derived vasculature	Including femoral nerve stump in implant increases function 5-fold compared to no-stamp implants	[311]
Mouse	C2C12+Fibs+HUVECs	PLLA/PGLA	Subcutaneous, quadriceps and abdominal wall	N/A	N/A	Host and donor anastomosis	Tri-culture results in greastest vascularization	[302]
	C2C12+Fibs+HUVECs	PLLA/PGLA	Abdominal wall	N/A	~0.1 mN/ mm^2	Host and donor anastomosis	Tissues kept in culture for 3 weeks show better vsacular infiltration than those implanted after 1 day	[303]
	C2C12	Fibrin	Gluteus	N/A	N/A	Agrin promotes greater host vasculature infiltration	Pre-treatment with agrin promotes acetylcholine receptor clustering and innervation by host nerves	[236]
Human	MPCs	Porcine Bladder submucosa	Latissimus Dorsi	ND	0.9 ± 0.1 mN/mm^2	Not reported	Function only seen after cyclic stretch. Implant force increases from 2 to 4wks post implantation	[242]

MPCs, muscle progenitor cells; MSCs, mesenchymal stem cell; HUVECs, human umbilical vein endothelial cell; PLLA, poly-(L-lactic acid); PLGA, polylactic-co-glycolic acid; VML, volumetric muscle loss; N/A, not assessed; ~, estimated based on figure in paper

growth factors, such as IGF-1, resulting in enhanced muscle regeneration and innervation [315, 316]. Lastly, a recent approach involving the use of human mesangioblasts transduced with the proangiogenic placenta growth factor (PlGF) embedded in fibrinogen-PEG hydrogel restored a full murine *tibialis anterior* muscle defect yielding vascular and neuronal ingrowth into engineered muscle implants [317]. Mesangioblast implants not expressing PlGF showed decreased vascular infiltration, muscle size and density, suggesting that the proangiogenic effects of PlGF promoted survival of muscle fibers [317, 318]. While single explanted myofibers from the regenerated muscle had specific forces similar to those of native myofibers, it is unclear if they were purely of human origin and if contractile force from the whole regenerated muscle (reflective of functional recovery) was the same as that of the intact TA muscle.

Cardiac muscle

Similar to skeletal muscle, successful pre-vascularization of engineered cardiac tissues has typically required co- and/or tri-culture of cardiomyocytes and vascular cells, most commonly endothelial cells and either fibroblasts or smooth muscle cells [277, 286, 319–322] (reviewed in [323]). Beside adding supporting cell types, engineering cardiac tissues using porous scaffolds [184, 319, 324, 325] or impregnating scaffolds with pro-angiogenic factors such as VEGF [184, 326] or stromal-cell derived factor-1 (SCF-1) [326] have enhanced host neovasculogenesis upon tissue implantation in both normal and infarcted hearts. Hence, numerous studies have assessed the ability of engineered cardiac tissue implants to actively repair infarcted myocardium in small and large animal models (Table 2). While various studies have shown some degree of structural and functional repair, the results differ widely on the extent of functional recovery, graft vascularization, and electrical integration with the host heart. Of note, very few studies assessed in vitro contractile function of engineered tissues prior to their implantation, and no studies reported important measures of electrical function and electrical safety, such as conduction velocity. A recent comparison between injected hPSC-CMs and transplanted hPSC-CM patches showed that injected cells were able to form electrical connections with host myocardium [327] while functional integration of transplanted patches was hampered by the existence of fibrous layer between the patch and host myocardium. Thus, the lack of direct electromechanical integration between implant and host cardiomyocytes remains an important challenge in the field, resolution of which would be expected to further enhance therapeutic effects of engineered cardiac tissues beyond mere paracrine effects. Importantly Menasche et al. has recently presented the first clinical implantation of a hESC-derived cardiac progenitor patch into a human heart [328]. No adverse effects and preliminary evidence of significant improvement in the patient's heart failure severity offer encouraging prospects for the use of hPSC-derived cardiac tissues in human heart repair, and will undoubtedly be followed up by future clinical trials and in-depth mechanistic studies.

Future considerations

Successful therapies for striated muscle dysfunction will require further technological advances and improved understanding of muscle physiology, development, and disease. Congenital muscle pathologies are most likely to benefit from gene therapies that employ modern genome engineering techniques [342–345], while cell therapies with healthy or genetically corrected cells will be limited to repair of the heart and specific skeletal muscles, or potentially all striated muscles via intra-arterial delivery of mesoangioblasts [124, 126], The use of biomaterials with or without cells will likely be most beneficial for treatment of myocardial infarction [346] and traumatic skeletal muscle injuries such as volumetric muscle loss [168, 172].

One of the important challenges in the cell-based human skeletal muscle repair is the inability to expand satellite cells in sufficient quantities without loss of their myogenic potential [94]. Successful methods to recreate SC niche and enable SC self-renewal in vitro may require the presence of differentiated myofibers and various non-muscle cells (FAPs, ECs, pericytes), manipulation of ECM proteins and substrate mechanics, and use of 3D cell culture [75, 98, 212]. Alternatively, generating functional SCs from hPSCs may overcome this challenge but improved differentiation protocols, via identification of novel small molecules, cell sorting, or genome editing, will be required to increase the efficiency of myogenic hPSC differentiation. Furthermore, while hPSC-derived myogenic precursors have been shown to fuse with mouse myofibers and contribute injury repair in vivo, their ability to form functional human muscle is still unknown. For systemic delivery of myogenic cells it will be also important to promote their passing through capillary walls, which could be achieved by use of gene editing techniques (e.g. CRISPR/Cas9) to safely and stably express proteins such as Pw1/Peg3 [128].

In contrast to skeletal muscle, where implantation of muscle progenitors can contribute to formation of new mature myofibers and muscle repair, injection of immature hPSC-CMs in the heart may not lead to the formation of mature cardiac muscle and may predispose heart to life-threatening arrhythmias [162]. Tissue engineering strategies have emerged as alternative cell delivery methods

Table 2 Summary of in vivo cardiac muscle repair, vascularization, and integration via implantation of in vitro engineered tissues

Cell source	Cell types	Scaffold	Model	In vitro function	Electrical integration	Vascularization	Effects	References
Rat	NRVMs + ECs	Cell sheet	Rat sub-acute MI	N/A	N/A	Host-graft anastomoses	Higher EC density increased LVEF and capillary density and decreased scarring	[320]
	NRVMs	Cell sheet	Rat sub-acute MI	N/A	Yes	Factor VIII(+) cells with CMs	Improved cardiac function 2–8 weeks post implantation	[329]
	NRVMs	Cell sheet	Rat chronic MI	N/A	No	No	Improved cell viability and LV function relative to cell injection controls	[330]
	Fetal and neonatal CMs	Collagen I gel	Rat chronic MI	~2.2–4 mN/mm^2	No	Host-derived neovasculature	Fetal but not neonatal CMs improved systolic function, possibly via increased donor CM proliferation	[331]
	NRVMs	Collagen I gel	Rat chronic MI	~5–6 mN/mm^2	Yes	Host-derived neovasculature, primitive vessels	Improved systolic function and wall thickness, decreased dilation	[274]
	NRVMs	Fibrin gel	Rat acute MI	~4 mN/mm^2	No	Host-derived neovasculature,	Improved LVEF, decreased infarct size and free wall thinning	[332]
	NRVMs	Alginate gel	Rat sub-acute MI	N/A	Yes	Omentum-based donor vasculature and host vessels	NRVMs treated with IGF-1, SDF-1, VEGF and pre-vascularized on omentum prevent heart dilation	[326]
	NRVMs + rat skeletal myoblasts	Laminin-coated PGS	Mouse sub-acute MI	N/A	N/A	Host-derived neovasculature	Increased host cardiac strain ratios after implanting patches with VEGF-transduced myoblasts	[333]
	NRVMs	Matrigel/collagen	Rat chronic DCM	~0.8 mN/mm^2	Yes	Host-graft anastomoses	Improved FS, peak pressure generation and responsiveness to dobutamine	[334]
	Adipose- rat MSCs	Fibrin gel	Rat acute MI	N/A	N/A	Host-derived neovasculature	Improved LVEF and decreased LV remodeling compared to Fibrin gel alone	[335]
Mouse	miPSC-CMs	Cell sheet	Rat chronic MI	N/A	Possibly	N/A	Decreased fibrosis, improved ventricular function	[278]
	mESC-CMs +ECs +MCs	Cell sheet	Rat sub-acute MI	N/A	N/A	Host-derived neovasculature	Improved systolic function, decreased remodeling	[277]

Table 2 continued

Cell source	Cell types	Scaffold	Model	In vitro function	Electrical integration	Vascularization	Effects	References
Human	hiPSC-CMs +ECs +MCs	Cell sheet	Rat sub-acute MI	N/A	No	Host-derived neovasculature	Improved FS and systolic thickening 2-8wk post implant. Grafts 5–44 % of infarct area	[321]
	hiPSC-CMs	Cell sheet,	Rat sub-acute MI	N/A	No	N/A	Enhanced anterior wall thickness, poor graft size relative to injected cells	[327]
	hiPSC-CMs	Cell sheet	Pig sub-acute MI	N/A	N/A	Graft capillaries	Improved ventricular function, attenuated cardiac remodeling	[336]
	hESC-CMs	Collagen I gel	Rat chronic MI	~0.5 mN/mm^2	No	Host-derived neovasculature	Attenuated disease progression, enhanced Cx43 expression and sarcomeric alignment after 220 days	[283]
	hiPSC-CMs + ECs + SMCs	Fibrin patch + IGF-1	Pig acute MI	N/A	N/A	Host-derived neovasculature	Improved LV function, myocardial metabolism, arteriolar density when cells implanted with fibrin + IGF-1 patch	[161]
	hESC-CPC (SSEA1$^+$)	Fibrin gel	Human chronic MI	N/A	N/A	N/A	NYHA Class III to Class I improvement Previously akinetic regions now hypokinetic, 10 % increases in LVEF,	[328]
	hESC-CMs	Gelatin (Gelfoam)	Rat sub-acute MI	N/A	Possibly	N/A	Improved preservation of graft size with cyclic stretch pre-conditioning	[337]
	hESC-ECs and SMCs	Fibrin gel	Mouse and pig acute MI	N/A	N/A	Host-derived neovasculature/ primitive vessels	Improved LV function, reduced infarct size, recruitment of c-Kit$^+$ cells	[324, 338]
	hMSCs	Collagen I gel	Rat acute MI	N/A	N/A	Host-derived neovasculature	Increased anterior wall thickness and fractional shortening	[339]
	hUCB- MSCs	Fibrin gel	Mouse acute MI	N/A	N/A	Host-graft anastomoses	Cardiac differentiation of implanted cells, improved LVEF and fractional shortening	[340]

CM, cardiomyocyte; EC, endothelial cell; MC, mural cell; SMC, smooth muscle cell; NRVM, neonatal rat ventricular myocyte; mESC, mouse embryonic stem cell; miPSC, mouse induced pluripotent stem cell; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; hMSC, human mesenchymal stem cell; rMSC, rat mesenchymal stem cell; UCB, umbilical cord blood; HUVEC, human umbilical vein endothelial cell; MEF, mouse embryonic fibroblast; FA, fractional shortening; LVEF, left ventricular ejection fraction; FS, fractional shortening; PGS, poly(glycerol sebacate). MI, myocardial infarction; DCM, dilated cardiomyopathy; N/A, not assessed; ~, estimated based on reported tissue thickness

given their ability to improve cell retention and survival and to promote the maturation of hPSC-CMs, although even state-of-the-art 3D cardiac tissues still lack various properties of adult myocardium. It is possible that full maturation of engineered hPSC-CM tissues can be achieved through a combination of biochemical factors and electromechanical conditioning that closely mimic the complex microenvironment of developing myocardium. Moreover, understanding the complex interactions between hPSC-CMs and non-CMs (fibroblasts, endothelial cells, smooth muscle cells, pericytes, macrophages, etc.) will be likely required for the successful engineering of mature cardiac tissues. Alternatively, combinations of non-physiological stimuli may be identified that jump-start maturation programs in hPSC-CMs to accelerate their metabolic adaptation, hypertrophy, and acquisition of an adult functional phenotype.

For successful tissue engineering therapies, incorporating a vascular and neuronal component will be required to overcome the size and survival limitations. Obtaining robust vascular networks in vitro will likely require the presence of both endothelial and perivascular (pericyte, macrophage, smooth muscle, fibroblast) cells, yet the exact proportions of these cell types remains unclear. Furthermore, in vitro conditions for vascularization need to be identified that do not compromise differentiation, force generation, or electrical conduction (for cardiac) of engineered muscle. Related challenge is the inability to functionally integrate (i.e. enable synchronous activity) of engineered and host muscles in vivo. For engineered skeletal muscle, stable and functional NMJs are required to both promote muscle maturation and enable its innervation upon engraftment. *In vitro* or in vivo application of synaptogenic factors (e.g. agrin) [234, 236] or intermittent neuromuscular electrical stimulation [347] may be necessary to promote implanted engineered muscle survival, maturation, and formation of stable connections with the host motor neurons. For engineered cardiac muscle, the main contributor to the lack of electrical coupling between the implanted and host cardiac tissue is the fibrous layer that forms between the tissue implant and epicardium [327]. Surgical strategies to better adhere engineered tissues to the heart surface, or genetic engineering strategies to render the fibrous layer electrically conducting [348] may yield functional synchronization between the implanted and host cardiomyocytes.

Conclusions

Current pharmacological therapies show limited capacity to augment the regenerative response of human striated muscles to ischemic injury, excessive trauma, or degenerative disease. Over the past 35 years, a range of alternative cell-, gene-, and material-based strategies have evolved that have shown promising results in both small and large animal studies. Still, a greater understanding of the specific cellular and molecular mechanisms that can benefit striated muscle repair and regeneration is required to improve the efficacy of these approaches. For skeletal muscle therapies, improved cell isolation and culture techniques are needed to expand functional stem cells and maintain their myogenic potential in vitro. For effective cardiac repair, robust methods are required to accelerate in vitro cardiomyocyte maturation and promote transplanted cell survival in vivo. Finally, methods to produce large striated muscle tissues that can be rapidly vascularized and functionally integrated with host muscles need to be developed to enable successful translation of these promising strategies to clinical practice.

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