Translating musculoskeletal bioengineering into tissue regeneration therapies

Authors: *Alastair Khodabukus[1*], Tyler Guyer[2], Axel C. Moore[3,4], Molly M. Stevens,5[3*], Robert E. Guldberg[2*], and Nenad Bursac[1*]*

Affiliations:

[1]Department of Biomedical Engineering, Duke University; Durham, NC, 27708 USA.

[2]Knight Campus for Accelerating Scientific Impact, University of Oregon, Eugene, OR, 97403 USA.

[3]Department of Materials, Department of Bioengineering and Institute of Biomedical Engineering, Imperial College London; London, SW7 2AZ UK

[4]Department of Biomedical Engineering, University of Delaware; Newark, DE, 19716 USA

[5]Department of Medical Biochemistry and Biophysics, Karolinska Institute; Stockholm, 17177 SE

Corresponding authors. Email: nbursac@duke.edu (N.B.); aik12@duke.edu (A.K.); guldberg@uoregon.edu (R.E.G.); m.stevens@imperial.ac.uk (M.M.S.)

One Sentence Summary: This review discusses recent efforts in translating state-of-the-art bioengineering approaches to therapies for musculoskeletal regeneration.

Abstract

Musculoskeletal injuries and disorders are the leading cause of physical disability worldwide and a considerable socioeconomic burden. The lack of effective therapies has driven the development of novel bioengineering approaches that have recently started to gain clinical approvals. In this review, we first discuss the self-repair capacity of the musculoskeletal tissues and describe causes of musculoskeletal dysfunction. We then review the development of novel biomaterial, immunomodulatory, cellular, and gene therapies to treat musculoskeletal disorders. Lastly, we consider the recent regulatory changes and future areas of technological progress that can accelerate translation of these therapies to clinical practice.

INTRODUCTION

The musculoskeletal system is an interconnected multi-tissue system comprised of skeletal muscle, tendon, bone, ligament, and cartilage. These tissues collectively function to provide structural support, stability, form, and locomotion to mammals. With day-to-day activities, musculoskeletal tissues are subjected to various mechanical loads that can result in small lacerations and tears. Whereas tendon, ligament, and cartilage have limited self-repair capacity, skeletal muscle and bone can regenerate following minor injuries. However, larger or chronic insults can overwhelm the self-repair capacity of musculoskeletal tissues leading to a range of musculoskeletal disorders (MSDs). These disorders are characterized by tissue degeneration, functional decline, debilitating pain, disability, and even death.

MSDs affect 1.7 billion people worldwide (*1*) and can arise from traumatic injury, aging, autoimmune disease, or genetic mutations. Less severe MSDs are treated with physical rehabilitation and pharmaceuticals, while severe defects require surgical interventions including tissue grafting or the implantation of orthopedic devices. Autologous grafts (autografts) remain the gold-standard care for patients with severe MSDs, however, their use is hampered by donor site scarcity, morbidity, and pain. While cadaveric allografts or xenografts are frequently used to address the limited availability of autografts, they exhibit immunogenic risks and impaired tissue regeneration. Encouragingly, the use of non-biological orthopedic devices has shown increasing clinical success; yet, potential fibrotic response and suboptimal integration with host tissue can lead to graft failure long-term.

To overcome these limitations, researchers have been developing diverse bioengineering approaches towards new and improved therapies for musculoskeletal disorders. For example, advances in innovative bio-instructive and responsive biomaterials have led to the development of

next generation synthetic grafts and drug delivery systems that have shown promising results in animal models of MSDs (*2*). Improved methods to differentiate human induced pluripotent stem cells (hiPSCs) into various lineages and expand progenitor cells have opened doors to novel cell therapies with improved efficacy (*3-5*). The generation of more complex and biomimetic tissue-engineered equivalents holds the potential to produce patient-derived biological grafts and more clinically predictive drug screening platforms (*6, 7*). Recent advances in the gene therapy field have resulted in the first successful clinical trials for rare neuromuscular diseases (NMDs) (*8*). The promise of cell and gene therapies is further enhanced by the rapid advent of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology that provides unprecedented capability to precisely manipulate the human genome and epigenome (*9, 10*). Nevertheless, multiple hurdles and opportunities such as limited therapeutic efficacy, patient-specific responses, harnessing immune system capabilities, regulatory barriers, and high manufacturing costs must be overcome before widespread clinical use of these novel technologies.

In this review, we first discuss the intrinsic self-repair capacity of the musculoskeletal system. Next, we describe the causes of musculoskeletal dysfunctions and current state of patient care. We then review the contemporary bioengineering approaches to treat musculoskeletal disorders that are either recently approved for clinical use or in preclinical development. Lastly, we consider the avenues of future technological progress required to overcome the remaining barriers to translating these novel bioengineering therapies into clinical reality.

MUSCULOSKELETAL REGENERATION

Regeneration of musculoskeletal tissues critically depends on the ability of the innate and adaptive immune systems to orchestrate processes of: (i) damaged tissue clearance, (ii) expansion of tissue-specific progenitor cells, and (iii) tissue repair, remodeling, and/or de novo tissue formation (**Fig. 1A**). Regeneration is initiated by release of damage-associated molecular pattern molecules (DAMPS), chemokines, and lipid mediators from damaged cells to recruit neutrophils, monocytes/macrophages, and T-lymphocytes to the injury site (*11*). The recruited immune cells initially phagocytose cellular debris and) secrete multiple cytokines [e.g., interleukin 1α (IL-lα) and tumor necrosis factor a (TNFα)] to induce a pro-inflammatory environment that recruits additional immune cells and stimulates resident stem cell proliferation for subsequent tissue repair (*12*). Specifically, this stage of regenerative response is associated with pro-inflammatory transition of macrophages from an M0 to M1 phenotype and accumulation of $CD8^+$ and $CD4^+$ T helper 1 (T_h1) and T_h17 T cells (*11*). The final stage of tissue regeneration, tissue repair and remodeling, is characterized by loss of pro-inflammatory immune cells and accumulation of anti-inflammatory M2 macrophages and T cells (i.e., $CD4^+$ T_h2 and T_{regs}). These cells secrete anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 to repress the local inflammatory response and support tissue repair, remodeling, and/or de novo tissue formation (*12*).

Adult skeletal muscle regeneration is dependent upon resident muscle stem cells, named satellite cells (SCs), which inhabit a complex stem cell niche underneath the myofiber basal lamina (*13*) (**Fig. 1B**). SC fate is regulated by expression of the paired-box transcription factor 7 (PAX7) and the myoblast determination protein (MYOD1). Upon injury, SCs transition from being quiescent ($PAX7^+/MYOD^-$) to becoming activated, proliferative SCs ($PAX7^+/MYOD^+$). Activated SCs either return to quiescence for future rounds of muscle regeneration, or commit to differentiation

by loss of Pax7 and then either fuse into damaged/regenerating myofibers or form de novo myofibers (*13*). SC activation and proliferation is stimulated by pro-inflammatory cytokines and release of extracellular matrix (ECM)-sequestered hepatocyte growth factor (HGF) and fibroblast growth factor 2 (FGF2) (*13*). SC differentiation and muscle fiber formation is triggered by a shift to an anti-inflammatory immune response and subsequent proliferation of fibro-adipogenic progenitor cells (FAPs) in response to IL-4 (*14*). FAPs are muscle resident multipotent mesenchymal progenitor cells that can differentiate into fibroblasts, adipocytes, and possibly osteoblasts and chondrocytes (*15, 16*). FAPs support muscle regeneration by secreting ECM proteins (*15, 17*) and cytokines that regulate muscle formation (*15, 18*) and the inflammatory microenvironment (*19*). Perturbed inflammatory responses result in excessive FAP accumulation and subsequent fibrosis and adipogenesis, which are hallmarks of impaired muscle regeneration (*16-18*).

Adult bone also undergoes healing upon substantial fracture via a four-step process (*20*) (**Fig. 1C**). First, hematoma formation around the fracture site results in clearance of necrotic debris and recruitment of immune cells. The initial pro-inflammatory microenvironment stimulates resident osteogenic progenitor cell proliferation and recruitment of circulating mesenchymal stem cells (MSCs) to the injury site. Second, MSCs differentiate into chondrocytes to form soft fibrocartilaginous calluses to stabilize the fracture. Third, the cartilage tissue is subsequently remodeled and replaced with bone to form a hard callus. Fourth, a long period of bone remodeling begins which ultimately restores the original geometry and mechanical properties of the bone. Proper execution of this healing process requires four key criteria, collectively referred to as the 'diamond concept' for fracture healing (*21*). These criteria include: cells with osteogenic potential, an osteoconductive matrix, osteoinductive mediators, and mechanical stability. Bioengineering

therapeutic approaches aimed at improving or restoring bone regeneration therefore augment one or more of these factors. Mulitple growth factors serve as osteoinductive mediators across these steps. Fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), transforming growth factor beta (TGFβ), and bone morphogenetic proteins (BMPs) support recruitment, proliferation, and differentiation of osteoprogenitor cells.

While muscle and bone possess high regenerative capacity, adult ligament, tendon, and articular cartilage (AC) are much less regenerative, despite initiating a typical wound healing response to tissue damage. Ligament and tendon repair is characterized by an initial inflammatory response triggered by rupture of tendon vessels, hematoma formation, and infiltration of inflammatory cells (*22*). The inflammatory signals lead to activation and proliferation of resident tendon stem/progenitor cells (TSPCs), which secrete type III collagen-rich ECM. While this immature matrix initially supports rapid structural repair and neovasculogenesis, it fails to remodel long-term and is partially replaced by Type I collagen. The resulting fibrous tissue has inferior biomechanical properties compared to healthy tissue and is more prone to subsequent re-injury (*22*). In AC, DAMPs secreted by injured cells trigger proliferation and migration of cartilage-derived progenitor cells (CPCs) and other joint-resident MSCs (*23*). However, migration of these cells to the site of injury is limited by the dense cartilaginous ECM network, while the lack of vasculature further delays reparative immune response which has to rely on the diffusion of nutrients and signaling factors (**Fig. 1D**). Together, the lack of robust tissue repair creates a long-term pro-inflammatory microenvironment characterized by high concentrations of TNFα and IL-lα, which inhibit chondrocyte proliferation and differentiation (*23*, *24*). Additionally, chronic increase in reactive oxygen species and nitric oxide induces chondrocyte senescence and ECM degradation, leading to progressive cartilage degeneration (*25*).

BIOENGINEERING THERAPEUTIC APPROACHES

Biomaterial therapies

Biomaterial-based approaches to musculoskeletal tissue repair hold great promise as the mainstay therapy in the future. Currently, the most clinically advanced and utilized biomaterials do not contain live cellular components due to ease of regulatory approval, lower cost, and ability to be commercialized as off-the-shelf products. Based on their source, biomaterials can be classified as synthetic or naturally derived. Synthetic biomaterials such as organic and inorganic polymers, metals, or ceramics permit greatest control over structural design, mechanical properties, and degradation rates. However, they lack the complex biological cues found within natural biomaterials that increase regenerative and translational potential. Natural biomaterials are typically purified ECM proteins (e.g., collagen, fibrin, and laminin) or polysaccharides (e.g., hyaluronan, chitosan, and alginate) that have high biological activity but lack mechanical strength. The simplest biomaterial-based therapies for musculoskeletal tissue repair provide mechanical support but limited biological guidance cues and can induce sustained pro-inflammatory responses via the foreign body response. For implantable scaffolds used in articular cartilage repair, such as TruFit CB [composed of poly(lactic-co-glycolic acid) (PLGA) and calcium sulphate], this has resulted in poor long-term clinical outcomes and the need for revision surgeries (*26*). More advanced biomaterial strategies provide not only structural support but also biomechanical guidance cues or bioactive signals to augment native tissue regeneration or immune responses. For example, viscosupplementation typically utilizes hyaluronic acid to both enhance the rheological properties of synovial fluid and promote an anti-inflammatory response in damaged cartilage (*27*).

Alternatively, biomaterials can be used to deliver progenitor cells in biomimetic stem cell niches or to fabricate differentiated tissue-engineered biomimetic equivalents for transplantation.

Bioinductive scaffolds

For clinical success, biomaterial scaffolds for musculoskeletal regeneration need to be bioinductive to promote cellular infiltration, tissue remodeling, and long-term mechanical stability. Within such scaffolds, tissue-specific microenvironments can be created by tailoring biophysical characteristics including stiffness, microstructure, porosity, and degradation. Specifically, biomechanics of polymer scaffolds can be controlled by choice of monomer, molecular weight, polydispersity, crosslinking, blending, and use of interpenetrating networks (**Fig. 2A**) (*2*), with resulting bulk stiffness being of utmost importance to ensure the implanted scaffold can withstand anticipated biomechanical loads. Since the local tissue stiffness regulates stem cell fate and differentiation (*28*), material choice and processing should be carefully chosen based on the tissue of interest: skeletal muscle (~10-20 kPa), cartilage (~1-10 MPa), and bone (~1-20 GPa). Scaffold porosity can be further tuned to enhance cellular infiltration, vascularization, and mass transport, but at the expense of mechanical stiffness. This trade-off can be minimized by modifying surface topography and chemistry to direct cell fate and differentiation independent of stiffness (*29*). Because musculoskeletal tissue interfaces such as the myotendinous junction and osteochondral unit are most prone to failure, interfacial scaffolds can be designed to support multi-tissue repair with long-term therapeutic benefit. These scaffolds are composed of tissue-specific phases/units that provide optimal biomechanical and bio-instructive properties to support cell-specific differentiation (*30*), including graded transitions in tissue structure and mechanics. Triphasic scaffolds such as MaioRegen, comprised of different ratios of type I collagen and

hydroxyapatite organized into three layers, have successfully treated osteochondral defects in clinics *(31)*.

An additional design consideration is the creation of tissue-specific stem cell niches to guide stem cell fate and tissue formation, which can be achieved via use of specific ECM composition, cell adhesion moieties, topological cues, and morphogen tethering. Minimally processed native ECM proteins, such as laminin, collagen, and fibronectin, have favorable characteristics for cell adhesion, growth, and differentiation and need to be incorporated into synthetic materials to achieve the same effects. Batch-to-batch variability of native proteins can be decreased by use of synthetic cell adhesion peptides (e.g., RGD, IKVAV) (*32*). Additionally incorporation of recombinant integrins such as α4β1 or α3/α5β3 along with niche-specific ECM proteins can be used to maintain SC quiescence (*33*) or promote osteogenic differentiation (*34*), respectively. Cell behavior and tissue growth can be further influenced by regulating scaffold features such as shape, aspect ratio, and curvature to control local topography (*35, 36*). Finally, the ideal biomaterial should be fully biodegradable and gradually replaced with regenerating tissue without loss of mechanical stability. The innate degradation and remodeling potential of natural materials can be modified through the formation of crosslinks, functionalization of active side groups, or incorporation of protease inhibitors. Synthetic polymers can be made biodegradable (e.g., polyesters) and tuned to meet the desired tissue regeneration rates via use of copolymers, polymer blends, enzyme cleavable bonds, or cell-mediated release of degradation products that additionally support tissue repair [e.g., liberated Ca^{2+} or PO_4^{2-} ions from tricalcium phosphate (TCP) that stimulate osteogenesis (*37*)].

An alternative bioinductive scaffold can be derived by chemically and enzymatically digesting organs to remove cellular material and generate decellularized ECM (dECM) (**Fig. 2B**). While

decellularization can alter tissue architecture, these scaffolds retain ECM proteins and biological cues such as cell binding motifs, growth factors, ECM-modifying enzymes, and matrix-bound nanovesicles (MBVs), which can direct anti-inflammatory and pro-regenerative immune and cellular responses upon implantation (*38*). Preserved tissue specificity of bioactive cues in dECM scaffolds can be further leveraged to guide tissue-specific biological programs both in vitro and in vivo *(39)*. Because native architecture is maintained, dECMs can be implanted as biomimetic scaffolds that provide guidance cues for tissue growth and neurovascular integration. For example, dECM cancellous bone scaffolds coated with collagen-hydroxyapatite composites have been applied to robustly enhance osteogenesis (*40*). Alternatively, dECM can be processed into sheets, coating materials, or injectable hydrogels for in vivo or in vitro applications (*41*). When implanted, dECMs induce biomimetic pro- and then anti-inflammatory responses that promote cellular recruitment and tissue formation (*42*), although immune response type and strength are tissue-specific and inherently variable (*39*). In a small clinical trial of 13 patients, dECM sheet implantation in combination with physical therapy was shown to support small increases in muscle mass and strength in a subset of patients with long-term volumetric muscle loss (VML) (*7, 43*). Nevertheless, the main long-term clinical use of dECMs will likely involve coating of synthetic implants to promote cell adhesion and cell/tissue delivery (*41*).

Growth factors and platelet rich plasma

Systemic or local delivery of soluble growth factors leads to their rapid degradation and loss of bioactivity, resulting in limited clinical benefits (*44*). Alternatively, growth factors can be conjugated to biodegradable biomaterials and their release profile controlled by regulation of biomaterial degradation rate. In skeletal muscle, SC proliferation or hypertrophy have been

stimulated by sustained biomaterial release of FGF2 (*45*), HGF (*46*), or insulin-like growth factor I (IGF-I) (*45*). Alternatively, SC proliferation has been stimulated by nanoparticle delivery of the small molecule drugs CEP-701 (*47*) or combined forskolin and RepSox delivery (*48*). In 2002, the food and drug administration (FDA) approved Infuse, a collagen sponge containing BMP-2, for treatment of long bone fractures, non-unions, and spinal fusions. However, multiple side-effects including ectopic bone formation, osteoclast activation, bone-cyst formation, and inflammatory complications have been reported (*49*). Alternative delivery scaffolds and additional growth factors or bisphosphonates have been used to replace BMP-2 or minimize its side effects (*49, 50*). Due to its important roles in tissue repair, vascularization has been stimulated in studies by delivery of proangiogenic factors such as vascular endothelial growth factor (VEGF) and platelet derived growth factor BB (PDGF-BB). Temporally regulated release of VEGF followed by PDGF, in particular, has resulted in the formation of stable mature vessels (*51*). Additionally, combined delivery of angiogenic and myogenic (*52*) or osteogenic (*53*) growth factors can synergistically augment tissue regeneration.

Articular cartilage defects in small animal models have been repaired utilizing IGF-I and/or members of the TGF-β, BMP and FGF families to stimulate chondrocyte proliferation and differentiation, ECM synthesis, and to decrease the catabolic actions of IL-1 and matrix metalloproteinases (*54*). However, the dense and highly negatively charged cartilaginous ECM network restricts growth factor diffusion and penetration into deeper cartilage areas, limiting successful translation to larger-sized human defects (*55*). These size and charge limitations can be overcome by use of cationic nanoparticle delivery vehicles such as polyamidoamine dendrimers (*56*) and avidin (*57*) to enable successful growth factor delivery into human-sized cartilage defects.

Since robust tissue repair requires the presence of multiple growth factors, delivery of single or select factors may be insufficient for complete regeneration. For example, intra-articular injections of recombinant human FGF-18 in patients with knee osteoarthritis failed to improve primary study outcomes (*55*). As such, the high growth factor content of platelet-secreted α-granules has been used to stimulate tissue regeneration by injections of centrifugation-concentrated platelet rich plasma (PRP) (*58*). Alternatively, sonication or freeze-thawing of PRP to generate a cell-free platelet lysate (PL) containing an undefined cocktail of multiple growth factors (e.g., IGF-1, VEGF, and EGF) and cytokines (e.g. IL-6, IL8, and TNFα) has also been utilized as an autologous pro-regenerative therapy in multiple pathological settings (*58*). To date, despite promising small clinical studies of skeletal muscle (*59*), bone (*60*), and cartilage (*61*) repair, there has been insufficient evidence to support the wide utility of PRP or PL as a regenerative therapy (*62*). However, it is possible that sustained and regulated PRP release via polymeric conjugation could increase the therapeutic efficacy of this approach, as observed in wound healing applications (*63*). Additionally, supplementing or depleting specific growth factors within PRP could yield more substantial and reproducible regenerative responses (*64*).

Immunomodulatory biomaterials

Due to the pivotal roles of the immune system in regulating tissue regeneration, immunomodulation has become an attractive strategy to induce and control tissue repair. Clinically, autoinflammatory and autoimmune diseases have been traditionally treated with various immunosuppressants. However, chronic and broad immunosuppression results in suboptimal regenerative response and increased risk of opportunistic infections (*65*). Next-generation corticosteroids with increased specificity and decreased side effects, such as vamorolone, could improve regenerative response of muscle tissue, as seen in recent clinical trials

in patients with Duchenne muscular dystrophy (DMD) (*66*). Alternatively, specific targeting of immune cells such as myeloid-derived suppressor cells (MDSCs), which suppress T and B cell responses and correlate with impaired bone healing in mice (*67*), may lead to more clinically successful systemic immunotherapies. To circumvent the complexities of modulating systemic immune responses, a range of immunomodulatory biomaterials have been developed to enhance regenerative outcomes. Historically, the desired immunomodulatory trait for implanted biomaterials has been the suppression of immune response to prevent foreign body response. However, it has become evident that an acute pro-inflammatory phase following trauma characterized by an M1 macrophage response is beneficial or even necessary for regeneration (*68*). Muscle regeneration, for example, can be accelerated by amplifying the M1 pro-inflammatory cascade with the addition of M1, but not M0, macrophages (*69, 70*), or by transient overexpression of granulocyte-macrophage colony-stimulating factor (GM-CSF) (*71*), both of which result in increased SC proliferation. Nevertheless, M2 macrophage response is also necessary to complete tissue regeneration and healing response (*72*). Temporal modulation of immune responses by inducing biomimetic short- and long-term pro- and anti-inflammatory responses, respectively, may be optimal for augmenting endogenous or implant-induced tissue regeneration. Specific immune responses can be stimulated by adjusting physicochemical properties of biomaterials such as surface charge, wettability, and porosity. For example, anionic and hydrophilic surfaces inhibit monocyte adhesion and promote anti-inflammatory responses, whereas cationic and hydrophobic surfaces promote monocyte adhesion and inflammatory signals (*73, 74*). Larger biomaterial pore sizes have been shown to correlate with increased M2 macrophage polarization compared with smaller pore sizes (*75*). Softer substrates and topographies that induce a more elongated cell shape can promote M2 polarization. Certain materials such as low molecular weight xanthan gum (*76*)

and squid-derived collagen type II (*77*) show chondroprotective anti-inflammatory properties. Additionally, biomaterials can serve as drug-release vehicles to modulate immune responses. For example, IL-4-loaded gold nanoparticles stimulate M2 immune responses and promote muscle regeneration and contractile force recovery in acute injury (*78*) and DMD (*79*) mouse models.

CELLULAR THERAPIES

While acellular therapies can promote cellular infiltration and augment tissue regeneration, they have limited capacity to restore substantial cell loss in critically sized defects. Cell-based therapies, on the other hand, can provide cellular material for tissue formation and/or secreted paracrine factors to augment endogenous regenerative response (**Fig. 3)**. Cell based therapies typically utilize primary progenitor cells or cells derived from hiPSCs.

Primary cell therapy

Primary progenitor cells have limited expansion potential in vitro and their extended culture can induce permanent alterations, leading to reduced therapeutic potential (**Fig. 3A**). The two main primary cell types used in musculoskeletal therapies have been tissue-resident progenitor cells and MSCs. Specifically, in vitro expanded SCs have long held promise for treatment of NMDs and VML. However, their clinical use in DMD patients was unsuccessful due to poor cell survival, motility, and engraftment/fusion with host myofibers (*80*). Poor SC engraftment was largely attributed to traditional in vitro culture that yielded spontaneous activation and rapid loss of PAX7 expression in SCs, caused by their displacement from the in vivo niche (*81*). Therefore, a clinically relevant expansion protocol for SCs would need to maintain PAX7 expression and prevent activation, and/or to deactivate SCs prior to transplantation. Culture with pro-inflammatory cytokines and small molecules (*82, 83*) and use of soft culture substrates with muscle-like stiffness (*83, 84*) have shown some success with mouse SCs, although promising results with human SCs

are yet to be demonstrated. Similarly, successful deactivation or return to quiescence of expanded SCs has not been achieved, though SC quiescence can be maintained in a complex media at the expense of limited cell expansion (*33*).

For the last 30 years, autologous chondrocyte implantation (ACI) has been clinically utilized to treat focal cartilage defects (*85*). In vitro-expanded chondrocytes are injected into the defect and retained at the site of injection by a periosteal flap or more recently by a collagen or synthetic membrane. However, extensive chondrocyte expansion in vitro results in cell dedifferentiation characterized by loss of chondrogenic gene expression and adoption of a fibroblastic morphology and gene expression (*86*). Encouragingly, extensively passaged chondrocytes can regain chondrogenic potential by acute 7-day rejuvenation culture in three-dimensional (3D) aggregates in media supplemented with chondrogenic growth factors, the glycosaminoglycan-degrading enzyme chondroitinase-ABC, and collagen crosslinker lysyl oxidase-like 2 (*87*). Lastly, multipotent MSCs, which can differentiate into bone, cartilage, and adipose tissue, have been trialed extensively in patients over the last 25 years. Direct MSC injection can be conducted with minimally invasive techniques and has shown promise in small clinical trials for treatment of delayed and non-union fractures (*88*). To date, at least 10 MSC therapies have been approved worldwide, though not by the FDA (*89*). The clinical efficacy of MSC therapies has been variable due to divergent cell culture procedures and loss of MSC therapeutic potential with passaging. Similar to muscle progenitors, expanding MSCs on soft hydrogel substrates can promote their stemness leading to improved therapeutic potential (*90*).

hiPSC-based therapy

Shortcomings of primary cell expansion can be overcome by using hiPSCs, which continuously expand and can be differentiated into any somatic cell type. State-of-the-art hiPSC-derived muscle

progenitor cells (iMPCs) are generated via directed differentiation methods that yield heterogeneous cell population of SC-like cells, activated SCs, and differentiated myotubes (*91*). While various cell surface markers have been identified to purify iMPC subpopulations with increased therapeutic potential (*91, 92*), these cells still have 50 to 60-fold lower engraftment efficiency than native SCs, do not always localize to the SC niche, and transcriptionally resemble fetal myoblasts (*5, 93*). Encouragingly, 4 weeks after implantation, engrafted iMPCs adopted a more adult-like SC transcriptome and when reimplanted engraft with 20-fold higher efficiency, suggesting that the in vivo microenvironment enhances iMPC maturity and function (*93*). Osteoblasts and chondrocytes can be obtained from hiPSC-derived MSCs (iMSCs) which exhibit tri-lineage differentiation potential, albeit with lower adipogenic potential than primary MSCs. Encouragingly, compared to primary MSCs, iMSCs have increased expansion potential and rejuvenation molecular signature, and can successfully treat critical-size porcine bone defects (*3, 94*). Osteoclasts can be derived from hiPSC-derived macrophages and stimulate mature bone formation in vitro and in vivo when cultured with iMSCs (*95*). The use of single-cell RNA sequencing and CRISPR-Cas9 driven fluorescent reporters can allow identification and purification of osteoblasts (*4, 96*) and chondrocytes (*97, 98*) with increased differentiation and therapeutic potential. Nevertheless, generation of adult-like mature cells and tissues from hiPSCs remains an important challenge (**Fig. 3B**). Additionally, long-term safety of hiPSC therapies requires the elimination of their tumorigenic potential by ensuring use of non-integrating reprogramming factors, uniform and robust differentiation protocols, and identification and removal of pluripotent or immature, proliferating cells (*99*). Specifically, generation of hiPSC lines with drug-inducible suicide genes can be utilized to partially or fully eradicate transplanted cells in cases of adverse outcomes in vivo (*100*).

Cell delivery

Cells can be delivered to the site of tissue injury via localized injection or systemic delivery. Systemic cell delivery prevents the need for surgical interventions but requires that cells cross the endothelial barrier and home to the injury site. As SCs cannot cross blood vessel walls, systemic cell therapies for NMDs have focused on intra-arterial delivery of $CD133^+$ stem cells and mesoangioblasts, blood vessel-associated progenitor cells with myogenic potential. However, despite promising mouse studies, phase 1 clinical trials with these cells failed to improve muscle function and only resulted in rare detectable dystrophin myofibers in a single DMD patient (*101*, *102*). Development of systemic cell transplantation therapies for NMDs and MSDs will require considerable optimization to increase engraftment efficacy and minimize cell sequestration by filtering organs. Some progress in this area has been made with improving homing of MSCs to sites of inflammation via use of small molecules (*103*), growth factors (*104*), ECM proteins (*105*), hypoxia (*106*), or genetic manipulations (*107*).

Additionally, survival and retention of implanted cells can be improved by their encapsulation in biomaterials to reduce shear stress and provide anti-apoptotic and pro-regenerative signals. For example, repair of murine VML has been facilitated by delivery of mesoangioblasts encapsulated in a polyethylene glycol-fibrinogen hydrogel (*108*), C2C12 cells on ultrathin PLGA ribbons (*109*), or SCs on collagen fibers coated with recombinant laminin and $\alpha4\beta1$ integrin mimicking the native SC niche (*33*). In 2016, the matrix-induced autologous chondrocyte implantation (MACI) technique was approved by the FDA (*110*). Like ACI, MACI utilizes expanded autologous chondrocytes but transplants them on a porcine collagen type I-III membrane rather than within a cell suspension. The MACI procedure can be performed arthroscopically and with fibrin glue to

minimize vasculogenic hypertrophy, which further improves clinical outcome compared to ACI and is suggested to be capable of treating defects >2 cm^2.

The success of cell therapies critically depends on immune matching between the implanted cells and host patient. Autologous cell therapies circumvent this issue but time and cost to generate therapeutically relevant cell quantities are often prohibitive (*111*). Allogenic cell therapies require human leukocyte antigen (HLA) matching to minimize adverse immune reactions but may still require immunosuppression. Alternatively, HLA cloaking, where specific HLA isoforms are deleted using CRISPR-Cas9 technology, could theoretically allow generation of a small number of donor cell lines that are immunocompatible with most of the world's population *(112)*. MSCs are hypoimmunogenic due to the lack of class II HLA and co-stimulatory molecule expression required for T cell activation (*89*). Additionally, MSCs have highly potent immunomodulatory and immune-dampening properties via cell-cell contact and paracrine action, which contribute to their regenerative potential and broad applicability (*89*). However, MSC immunoprivilege can be lost following differentiation in vitro or in vivo, resulting in cellular cytotoxicity and immune rejection (*113*). Myoblast cell therapies can be augmented by the incorporation of macrophages that promote cell survival, proliferation, and migration (*114*). Similarly, incorporation of macrophages within engineered rat muscle tissues supports both in vitro muscle regeneration and in vivo survival (*115*).

Tissue-engineering approaches

Another cell-based strategy to treat musculoskeletal defects transplantation of functionally mature replacement tissues engineered in vitro using 3D scaffold or scaffold-free approaches (**Fig. 3C**). Scaffold approaches provide structural support and mechanical guidance cues to stimulate cell growth and tissue formation. Scaffold-free approaches rely on cell-generated ECM to support tissue development and include: cell sheets, aggregates/spheroids, and self-assembled tissues. Cell

sheets are usually formed by seeding cells on ECM-coated monolayers (*116*) or using thermoresponsive polymers, such as poly(N-isopropylacrylamide), which detach from culture plates upon decreased temperature (*117*). Scaffold-free tissues are inherently thin but can be formed into thicker constructs by rolling or stacking. Aggregates/spheroids can be formed by multiple methods including hanging drop cultures, microfluidics, and application of rotational forces to suspended cells (*118*), whereas self-assembled tissues are made by cell seeding at high density on non-adherent surfaces followed by tissue condensation (*119*). Compared to aggregate/spheroid cultures, self-assembled tissues can be reproducibly shaped into larger tissues with specific geometries. In the case of cartilage, the high cellularity of self-assembled tissues encourages integration into host tissue (*120*) and prevents stress-shielding that occurs in scaffold-based constructs that impedes matrix remodeling and synthesis (*121*). Muscle tissues also require high cell density and can form within soft mechanical microenvironments provided by either scaffold (*122-124*) or scaffold-free (*116, 125*) approaches. Recreating native hierarchical bone architecture, on the other hand, typically necessitates use of scaffolds reinforced with ceramics, such as hydroxyapatite and TCP, to ensure sufficient mechanical strength (*126*). Tissue-specific differentiation of MSCs can also be promoted by use of multi-phasic scaffolds and incorporation of specific growth factors such as HGF/IGF-1 (*127*), BMP-2 (*128, 129*), and TGF-β1 (*128*) or TGF-β3 (*129*) to promote muscle, bone, and cartilage differentiation, respectively. Once formed, tissue maturation and functionality can be increased by use of tissue-specific biophysical stimuli such as electrical stimulation (*130*), cyclic mechanical stretch (*131*), cyclical hydrostatic pressure (*132*), and compression loading (*133*). While adult-like function can be achieved with tissue-engineered bone and cartilage, gold-standard skeletal muscle tissues functionally and transcriptionally resemble embryonic to neonatal muscles.

For long-term clinical success, tissue-engineered muscle and bone implants must rapidly anastomose with the host neurovascular system to prevent cellular death and to facilitate seamless structural and functional integration between implant and host tissue. Vascularization is typically encouraged by either stimulating in vivo angiogenesis (i.e., host vessel ingrowth into the implant) or in vitro vasculogenesis (i.e., formation of vascular structures in the implant prior to transplantation). Angiogenesis in vivo can be stimulated using scaffolds with microgrooves (*134*), increased porosity (*135*) or surface roughness (*136*), but the rate of vascular ingrowth is typically insufficient to support survival of large grafts. To overcome this limitation, engineered tissue implants have been pre-vascularized in vitro by incorporation of vascular and supporting cell types (*124, 135, 137*). Increasing microvessel density and maturation through longer in vitro culture improves muscle implant perfusion, vascular density, and in vivo contractile function (*137*). Alternatively, thicker implants can be assembled by alternate stacking of muscle and vascular cell sheets (*117*).

Innervation of engineered tissues implants can be stimulated biochemically via application of soluble (*138, 139*) or biomaterial-conjugated (*140, 141*) agrin, which promotes myotube acetylcholine receptor clustering and neuromuscular junction (NMJ) formation. Similarly, use of magnesium-based alloys or bulk metallic glass bone implants induces secretion of sensory neuropeptides, such as calcitonin gene related peptide, to promote osteogenesis of periosteum-derived stem cells (*142*). Like angiogenesis-stimulating approaches, it is unlikely that biochemical stimulation will enable rapid innervation of large tissue implants. On the other hand, surgical neurotization increases innervation, neural integration, and regeneration of both muscle and bone implants but is therapeutically limited to small grafts (*143, 144*). This size limitation can theoretically be overcome by incorporation of neural progenitors to accelerate implant innervation.

For example, implantation of rodent or hiPSC-derived muscle tissues with incorporated motoneurons (MNs) promoted implant survival and NMJ formation, but did not support appreciable host neural integration (*138, 145*). Neural integration of implanted tissue can be further accelerated by the use of an engineered nerve conduit (ENC) to guide axonal growth toward the implant (*146*). In an ovine VML model, ENCs permitted functional innervation in 75% of implanted engineered muscle tissues and recovered force generation 3 months post-implantation (*125*). To date, this is the only preclinical animal model demonstrating the ability of in vitro engineered muscle tissues to restore large muscle defects.

More recently, advances in 3D bioprinting have enabled the generation of more defined and complex vascular and neural structures within 3D engineered tissues by sacrificial molding or direct cell bioprinting (*147*). Both methods have resulted in the formation of muscle tissues up to $1cm^3$, with incorporated vascular networks that anastomose with host vasculature and promote functional regeneration of VML injuries in rodents (*145, 148*). Alternatively, vascular ingrowth into thick 3D bioprinted tissues can be stimulated by use of porous bioinks leading to functional restoration after VML in mice (*149*). However, it is unclear if these approaches can be scaled from the $<1\ cm^3$ muscle volume to clinically relevant sizes for repair of human VML. 3D bioprinting can also be used to generate bone and cartilage tissues that mimic native cellular architecture (*148, 150*). However, current 3D bioprinting materials fail to match the stiffness of bone and cartilage, which will require development of novel bioink composites comprised of chemically modified synthetic and natural polymers (*150*). Lastly, a fundamental factor in developing a clinically successful musculoskeletal graft therapy will be the incorporation of physical activity and rehabilitation post-surgery, as shown in rodent models where graft functionality, vascularization, and functional innervation were increased by forced running (*151, 152*).

Organ-on-chip (OOC) platforms

Recent progress in muscle (*153*), bone (*154*), and cartilage (*155*) organ-on-chip (OOC) model systems has advanced our ability to study human musculoskeletal development, disease, and regeneration in vitro. Encouragingly, these tissue-engineered systems demonstrate expected physiological responses to pharmacological agents, showing promise for use in preclinical drug development studies. Additionally, multiple OOCs can be interfaced via microfluidic channels to enable unique studies of organ-organ crosstalk regulating musculoskeletal development and disease. Of particular interest are multi-tissue systems that anatomically diverge between mice and humans such as the NMJ and joints. Current human NMJ OOC models utilize compartmentalized chambers housing hiPSC-derived motor neurons and skeletal muscle tissues that enable visualization of neurite outgrowth and assessment of NMJ formation and function (*156-158*). While these systems mimic certain pathological features of NMDs such as impaired neuromuscular transmission in presence of myasthenia gravis patient serum (*157*), they lack maturation cues for achieving adult-like structure and function. For MSDs, joint-on-a-chip (JoC) systems that replicate native hierarchical structure and biomechanical loading hold potential for high-fidelity modeling of osteoarthritis (OA) and rheumatoid arthritis (RA) in vitro (*155*). Cartilage (*159*), subchondral bone (*154*), and synovial membrane (*160*) OOCs required for JoC systems have been already developed and utilized to study pathogenesis of OA and RA by applying hyper-physiological compression (*159*) or pro-inflammatory cytokines (*155, 160*). More complex, biomimetic JoC platforms will require additional incorporation of ligament, meniscus, Hoffa's fat pad, and neuromuscular OOCs (*155*). Overall, despite the fact that NMD (*156*) and OA (*159*) OOC models successfully replicate functional responses to drugs, more comprehensive studies will be needed to determine if they have a better clinical predictive value than traditional animal models .

GENE THERAPIES

Gene therapy approaches hold considerable potential to address various musculoskeletal diseases and deficits caused by genetic abnormalities, injuries, or aging. In the past two decades, rapid progress in the gene therapy field has led to initiation of more than 150 clinical trials (*161*). Multiple non-viral nucleic acid therapies such as antisense oligonucleotides (AONs) or plasmid gene deliveries have been developed to transiently modulate gene expression. The first clinically approved gene therapies for spinal muscular atrophy (SMA) and DMD have been exon skipping antisense oligonucleotide (AON) therapies. AONs are short (15-32 nucleotides) synthetic single-stranded nucleic acid sequences designed to bind and mask specific splice motifs resulting in the skipping of an exon (*162*). This results in restoration of the open reading frame and the generation of a truncated but partially functional protein (**Fig. 4A)**. To date, the FDA has granted accelerated approval to one AON for SMA as well as four AONs for DMD whereby skipping exons 45, 51, and 53 can together treat ~30-32% of patients. However, long-term follow up of eteplirsen showed low restoration of dystrophin protein that slows disease progression but is not curative (*163*). Current clinical trials (NCT04004065) for DMD utilize AONs with improved overall efficiency achieved by optimized molecular design (*164*) and conjugation to cell penetrating peptides (*165*). Overall, current AON therapies appear to have moderate benefit for patients and are costly due to short half-life of AONs requiring frequent re-administration.

Rather than AONs, it is likely that the long-lasting ex vivo and in vivo gene overexpression or genome editing approaches will become widely used for treatment of MSDs (**Fig. 4B**). Ex vivo approaches are cell-based and can permit sustained localized expression of therapeutic genes (e.g., growth factors) without the off-target effects associated with systemic delivery or burst release (**Fig. 4C**). Here, patient-derived cells are typically isolated and transduced with retroviral or

lentiviral vectors containing the gene of interest. In 2016, the European medicines agency approved the first ex vivo gene therapy, Strimvelis, which utilizes autologous $CD34^+$ cells retrovirally transduced with adenosine deaminase to treat severe combined immune deficiency (*166*). Additional approaches are aimed at modulating the inflammatory microenvironment to promote tissue regeneration by overexpression of cytokine genes such as *TGF-β1, TGF-β3, IL-6, IFN-β, IGF-I, BMPs, FGF-2, and VEGF-C* (*167*). Currently, the most clinically advanced gene therapy approach for cartilage is Invossa, where chondrocytes are transduced ex vivo to overexpress TGF-β1 and subsequently injected into the joint. Potential obstacles to this approach involve rapid clearance of injected cells and unintended attachment of cells on the synovial capsule rather than the articular cartilage. To overcome this obstacle, transduced cells can be embedded within 3D scaffolds to increase cell survival and retention at the implantation site (*168*). The feasibility of this approach has been shown in pigs where MSCs transduced with BMP2 and TGF-β3 embedded within decellularized bone matrices efficiently repaired full-thickness cartilage lesions (*169*). Additionally, aged muscle stem cells or OA chondrocytes can be rejuvenated in vitro by transient expression of Yamanaka factors, LIN28, and NANOG (*170*). When injected into injured muscle, rejuvenated mouse SCs restored aged muscle function to that of younger mice, suggesting potential to reverse age-related deficits in musculoskeletal regeneration and function.

Adeno-associated virus (AAV) therapy

In vivo gene therapies for MSDs most frequently utilize recombinant adeno-associated viruses (AAVs) which can induce stable and sustained gene expression as a single-dose therapy. Systemic AAV therapy is however hampered by the lack of tissue specificity (tropism), low transduction efficiency, and liver sequestration (*161*), which can lead to low efficacy, off-target toxicity, and the need for vector quantities that surpass current manufacturing abilities. Additionally, patients

may be ineligible for therapy due to pre-existing neutralizing antibodies or may develop strong immune responses to administered AAVs (*171*) or restored nascent protein, as seen with dystrophin protein expression in DMD patients (*172*). To overcome these challenges, novel AAV capsids with increased tissue tropism and transduction efficiency and decreased immunogenicity have been developed by directed evolution or rational design (*173, 174*). For example, novel myoAAVs require over 100-fold lower dose to exert therapeutic effects in muscle compared to current clinically utilized AAVs (*173, 174*). Similarly, AAV capsids can be engineered with tissue targeting peptides such as $(ASP)_{14}$ and $(AspSerSer)_6$ that target bone (*175*). Furthermore, immune responses to both AAV and nascent protein expression can be decreased by novel engineered AAV capsids (*176*), immunosuppression (*177*), or by treatment with DNA plasmid vaccines (*178*). Long-term clinical success may also require the ability of AAVs to successfully transduce stem cell populations that maintain tissue homeostasis. Encouragingly, efficient AAV transduction of SCs has been recently demonstrated which can support sustained muscle gene expression despite high myonuclei turnover (*174, 179*).

In 2019, Zolgensma, the first gene therapy for SMA, was approved for patients under the age of 2. This therapy is a one-time injection of AAV9 carrying the full copy of the *SMN1* gene required for motor neuron survival, and results in unprecedented patient survival and improved motor function (*8*). Unlike *SMN1*, dystrophin gene size (~14 kb) far surpasses the 4.7 kb packaging capacity of AAV, rendering gene therapy for DMD particularly challenging. Therefore, micro-dystrophin (μDys) constructs with less than 30% of the full gene length have been developed and were shown to improve skeletal and cardiac muscle function in preclinical non-human primate models of DMD (*177, 180*). Currently, three independent phase 1/2a trials are ongoing, with one showing dystrophin expression in ~80% of muscle fibers and sustained functional improvements

one year post treatment (*181*). Additionally, follistatin gene therapy to stimulate SC proliferation and muscle regeneration (*182*) has shown a good safety profile in phase 1 trials (*183, 184*). By promoting endogenous muscle regenerative potential, this approach can be used to treat both genetic and non-genetic causes of muscle loss and atrophy. For bone therapy, systemic AAV delivery of artificial microRNAs (miRNA), has been applied to modulate osteoblast and osteoclast activities and encourage bone formation in osteoporotic mice. Artificial miRNAs embed short hairpin RNA (shRNA) into miR-33-derived miRNA scaffolds to decrease shRNA mediated toxicity and off-target silencing. Specifically, downregulation of RANK or cathepsin K in osteoclasts (*175*) or Schnuri-3 (SHN3) in osteoblasts (*185*) enhanced bone formation and mechanical properties. While intravenous AAV delivery is suitable for disorders that impact all muscles or bones, the avascular nature of cartilage necessitates direct injection (*186*) or biomaterial-based delivery (*187*) of viruses for efficient transduction. For example, intra-articular injection of AAVs coding expression of IL-1 receptor antagonist (IL-1Ra), a physiological inhibitor of pro-inflammatory IL-1 signaling, has been proposed to slow or halt OA progression (*186*).

CRISPR-Cas9 therapy

Owing to rapid progress in the field, CRISPR-Cas9 genome editing therapies have already entered clinical trials (*188*). In its most basic form, CRISPR-Cas9 method employs guide RNAs (gRNAs) to direct a Cas9 endonuclease to create double stranded breaks (DSBs) at precise genomic locations. The DSB can be used for gene knockout by nonhomologous end joining (NHEJ), which results in random DNA insertions and deletions (indels) and subsequent nonsense-mediated mRNA decay. Alternatively, gene activation or insertion can occur by introducing a DNA sequence at the DSB by homology directed repair (**Fig. 4D**). While the efficiency of HDR is much

lower than NHEJ, it enables diverse genome editing outcomes with unprecedented precision. In preclinical studies, CRISPR-Cas9 therapy restored dystrophin expression and improved muscle contractile function in DMD dogs (*189*), and editing and safety were shown in parallel to persist for 18 months in mice - although off-target effects increased with time after therapy (*190*). CRISPR-Cas9 gene editing that constitutively upregulates BMP-9 has been used to stimulate osteogenic differentiation of iMSCs and enhance in vivo bone regeneration (*191*), although persistent expression and release of growth factors is expected to cause long-term side effects. In contrast, CRISPR-Cas9 insertion of TNFαR (*192*) or IL-1Ra (*192, 193*) in the inflammation-responsive chemokine (C-C) motif ligand 2 (CCl2) locus in implanted hiPSC-derived chondrocytes resulted in temporary, inflammation-dependent gene expression with improved therapeutic outcomes. Current work in the field is focused on increasing editing efficiency and decreasing potential off-target effects by use of Cas9 orthologues such as SaCas9 (*9*) to decrease Cas9 cargo size or CPF1 (*10*) to decrease off-target editing. The preferential systemic degradation of gRNAs is a main contributor to low editing efficiencies in vivo, which can be enhanced by increasing the gRNA to Cas9 ratio (*194*) and packaging gRNAs in a self-complementary (scAAV) rather than standard single-stranded AAV (ssAAV) (*195*). Additionally, the use of single-cut editing approaches (*196*) and screening of gRNAs in functional 3D tissues can further improve outcomes of CRISPR-Cas9 therapies (*197*). Together, rapid advances in the genome editing field hold great promise for curative therapies for a range of MSDs.

TRANSLATIONAL CHALLENGES AND FUTURE APPLICATIONS

Regulatory challenges

Historically, regulatory approval has been a slow process, contributing to the high cost of clinical product development and translation (*198*). Bioengineering approaches for musculoskeletal regeneration face considerable regulatory hurdles to clinical translation due to their frequent classification as combinations of devices, biologics, and drugs (*199*). Generally, devices have more rapid approval times than biologics and drugs (~6 years versus ~9 years versus ~11 years, respectively), which markedly influences commercial therapeutic design (*200*). Bioengineered devices for joint and cartilage replacement discussed in this review are likely to be regulated as Class III devices and require more lengthy premarket approval (PMA) based upon preclinical and clinical trial data. Cell and tissue-based therapies may be regulated under human cells, tissues, and cellular and tissue-based products (HCT/Ps) or under a biologics license application (BLA). The FDA requirements to qualify for HCT/Ps designation include minimal cell manipulation and homologous application [i.e., for the same basic function(s) as in the donor]. As such, musculoskeletal cells derived and/or expanded in vitro and genetically modified or incorporated into tissue-engineered products will require a BLA and will be classified as a device, biologic, or drug. First regulatory approvals have been recently received for modified cell therapies (e.g., chimeric antigen receptor T cell therapies) (*201*) and combined biomaterial and cell therapies (e.g. MACI) under BLA regulatory approval (*110*). Drugs under treatment or emergency classification (e.g., therapies treating small populations, such as monogenic diseases, or diseases requiring rapid treatments such as COVID-19) can receive accelerated approval after limited clinical trials.

To decrease regulatory burden multiple programs within the FDA (e.g., accelerated approval program, breakthrough therapy designation, and regenerative medicine advanced therapy

638 designation) and the European Medicines Agency (e.g., PRIME initiative) now exist to expedite
639 clinical translation of new regenerative therapies via a risk-based approach (*198, 202*). The impact
640 of these regulatory changes has been evident from the accelerated approval of gene therapies for
641 NMDs that would not be granted under previous regulations. For example, the clinical trial design
642 for the SMA gene therapy Zolgensma was streamlined by utilizing historical control cohorts due
643 to small patient numbers and leveraging the ethical issues associated with denying patients with a
644 low life expectancy (<2 years) (*202*). The Accelerated Approval Program decreases the threshold
645 for approval from demonstrating measurable clinical benefit to showing a surrogate endpoint that
646 predicts benefit for patients with severe disease and an unmet clinical need. This distinction
647 allowed four AON exon-skipping drugs for DMD patients to be approved based on demonstrated
648 dystrophin expression without a conclusive proof of a clinical benefit (*162*). While full approval
649 for these non-cellular therapies will still require demonstration of long-term safety and efficacy,
650 the new regulatory guidelines more rapidly grant patients access to potential life-extending or
651 saving treatments, while providing important feedback for new or improved product development.
652 However, it should be noted that accelerated approvals may result in commercialization of
653 therapies with increased safety risks, such as in the case of Class II devices with 510(k) approval
654 (*203*) where the device in question is only required to be equivalent to a preexisting approved
655 "predicate" device (*203*). While this should increase approved device safety profiles, further
656 refinements to PMA regulatory process are required to decrease development costs and promote
657 more rapid clinical translation of novel therapeutics.

658 ***Scale-up, manufacturing, and commercialization***

659 While the aforementioned regulatory changes are likely to expedite approvals of new
660 musculoskeletal therapies, substantial challenges with their scaling and commercialization remain.

To date, synthetic acellular biomaterials have been the subject of the most advanced methods for scale-up and manufacture due to lack of biological variability and existing experience with their clinical use. However, further product-specific developments to identify optimal sterilization techniques, ensure mechanical and structural reproducibility, and define pre-implantation and long-term quality standards will be required to achieve widespread clinical and commercial success. Likewise, the development of clinically utilized biological biomaterials will demand industry-wide regulations and procedural standardizations, such as those established by the FDA to generate dECMs with reproducible immune responses. Similar industry-wide standardization and regulatory oversight will be required for procedures and products that alter biomaterial structure and function, such as electrospinning and nanoparticle-based drug delivery carriers.

For cell-based therapies, efficient scale-up of stem cell production while retaining their therapeutic potential remains a key biological and technological challenge. Advances in understanding of stem cell biology, replicating in vivo tissue-specific niches with biomimetic scaffolds, and use of biochemical means to control stem cell fate and functional maturation will be critical for overcoming these barriers. Additional technological challenges are expected to arise when attempting to cost-effectively scale-up and automate multi-component self-renewal and differentiation culture systems (*204*). Equally important will be further infrastructural developments and regulatory guidance for the mass production, long-term cryogenic storage, and safe and timely delivery of cellular products. Due to associated complexities, widespread utility of personalized cell therapies will lag behind allogeneic cell use. The creation of allogeneic hiPSC and hiPSC-derived progenitor cell biobanks with characterized HLA haplotypes will follow the practices developed for bone marrow and cord blood biobanks. However, HLA matching does not guarantee immune privilege and necessitates immunosuppression in some patients. Alternatively,

HLA cloaking to generate a limited number of immunocompatible donor cell lines (*112*) would reduce total costs associated with hiPSC line derivation, line-specific differentiation, and the need for extensive pre-clinical validations. However, further optimization of HLA antigen expression and ensuring the absence of adverse off-target effects from CRISPR-Cas9 editing will be necessary. The most complex manufacturing and scale-up processes will need to be developed for multicomponent tissue-engineering therapies. In addition to the described requirements for biomaterial and cell-based therapies, tissue-engineered therapies will entail additional in vitro culture time, the incorporation of tissue-specific biophysical stimuli, and the use of multiple cell types leading to substantial increase in costs and challenges with quality control.

Scale-up of gene therapies to large numbers of patients will require substantial advances in AAV manufacturing capabilities to meet expected clinical demands. Further optimization of AAV and promoter design to increase tissue tropism and transgene expression while decreasing liver sequestration will decrease viral titers required for clinical efficacy. Alternative non-viral gene delivery approaches (e.g., use of nanoparticles) could overcome immune limitations associated with AAVs (*205*), with in vivo barcoding and directed evolution technologies serving to optimize polymer carrier blends for increased tissue tropism and transfection efficiency (*206*). For CRISPR-Cas9 and other genome engineering technologies, methods to rapidly identify optimal guide RNAs and increase editing efficacy will lead to decreased manufacturing costs. The last barrier to commercializing newly approved cell and gene therapies will be the establishment of national reimbursement policies, which so far have been hampered by the lack of cost-benefit analyses and

long-term efficacy data (*207*). However, ongoing longitudinal clinical studies and increased patient numbers are expected to produce viable strategies for reimbursement and commercialization.

While cell and gene therapies for musculoskeletal regeneration will encounter unique challenges before eventual commercial use, a key factor driving the cost of approved pharmacotherapies is their high failure rate in clinical trials (*208*). In vitro tissue-engineered human OOC systems hold promise to increase predictivity and decrease costs of preclinical drug development studies. To date, up to 10 distinct OOCs have been multiplexed to form a human-on-a-chip (HOC) platform (*6*) and successfully model known (and identify unknown) toxicities due to organ cross-talk (*209*). However, approaches to circumvent the Crabtree effect (*210*), for example by using physiological human plasma-like media (*211*), will be needed to accurately model human mitochondrial toxicity, metabolism, and drug responses. Additionally, incorporating more complex immune system-on-a-chip modules will account for roles of immune cells in tissue disease and regeneration (*212*). The industry-wide utilization of these platforms will further require that they can be automated, have non-destructive functional readouts, and are miniaturized to increase drug screening throughput (*153*). The modular nature of OOCs is suitable for modeling the complex musculoskeletal degeneration seen in multiple MSDs (*213*), and incorporating machine learning techniques during drug screening can allow accelerated development of combinatorial drug therapies at a fraction of the current cost. Despite their widespread use, preclinical murine models are limited by their small critical defects and poor modeling of human musculoskeletal structure, biomechanical loading, and immune responses, although mice with humanized immune system (*214*) can help address the latter issue. Large animal preclinical models thus remain the gold

standard for validating novel surgical therapies and the function of biomedical implants due to the ability to model human critical-size defects (*215*) and pathophysiology.

CONCLUSION

Over the last two decades, progress has been made in our ability to understand, model, harness, and augment endogenous tissue regenerative responses. Specifically, advances in biomaterial design, hiPSCs-based technologies, immunomodulation, OOC platforms, and machine learning have paved a way for the development of next-generation multi-component bioengineering therapies for musculoskeletal disease and dysfunction. The first approvals of such therapies in the past decade and continuous development of more streamlined regulatory guidelines will form a blueprint for rapid translation of successful preclinical studies into widespread clinical use. Together, we anticipate that in the next 10-20 years these advances will lead to a wave of new clinical therapies for MSDs.

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Conceptualization: AK, TG, ACM, MMS, REG, NB

Funding acquisition: MMS, REG, NB

Supervision: MMS, REG, NB

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Competing interests: MMS is an inventor on Patent Number US 9393097 B2 that describes an approach for repairing cartilage defects. All remaining authors declare that they have no competing interests.

Figure Legends

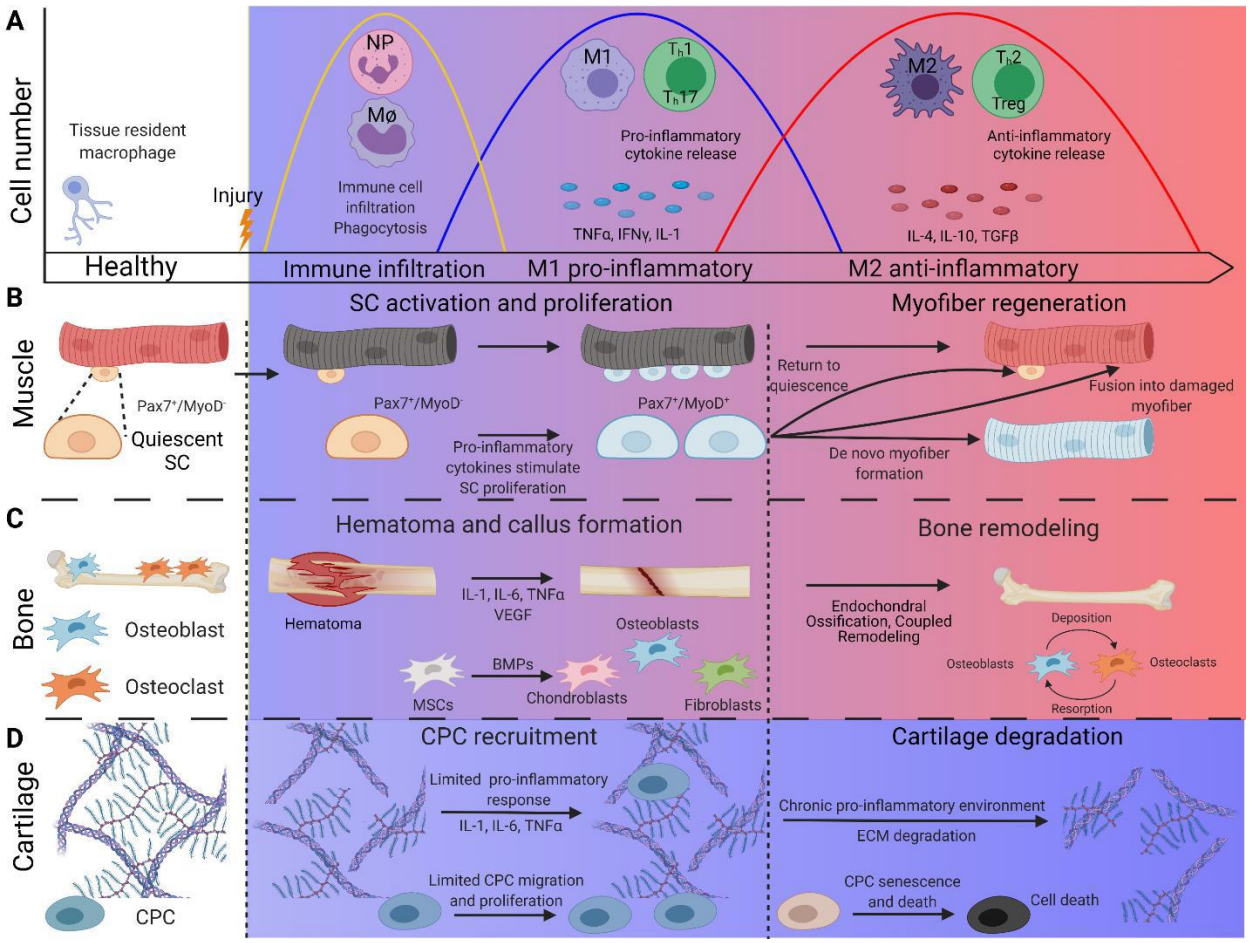

Fig. 1. **Musculoskeletal injury response.** Immune and tissue-specific progenitor cell regulation of musculoskeletal injury response in vivo. (**A**) Following injury, neutrophils (NP) and monocytes (M0) infiltrate the injury site to phagocytose damaged tissue and secrete factors that control fate of infiltrating immune cells. Initially, proliferation of immune and resident progenitor cells is stimulated by a pro-inflammatory microenvironment created by cytokine secretion from macrophages (M1) and T cells (T_h1 and T_h17). Subsequent tissue regeneration and remodeling are orchestrated by a switch to an anti-inflammatory microenvironment created by cytokine secretion from macrophages (M2) and T cells (T_{regs} and T_h2). (**B**) Skeletal muscle regeneration is orchestrated by muscle resident satellite cells (SCs) that in uninjured tissue are quiescent and

express the transcriptional factor PAX7. Upon injury, mechanical disruption and the pro-inflammatory microenvironment stimulate SC activation, proliferation, and MYOD expression. Activated SCs then fuse together to form de novo myofibers, fuse into regenerating myofibers, or return to quiescence by loss of MYOD. (**C**) Bone remodeling is characterized by an initial hematoma formation and pro-inflammatory microenvironment that recruits circulating MSCs. These MSCs initially differentiate into chondroblasts and fibroblasts to generate a fibrocartilaginous callous, which is further remodeled into bone tissue by MSC-derived and resident osteoblasts. Successful remodeling relies on the balanced synthetic and resorption activities of osteoblasts and osteoclasts, respectively. (**D**) In response to injury, cartilage undergoes a weak pro-inflammatory response that results in no-to-limited recruitment and proliferation of cartilage-derived progenitor cells (CPCs). Consequently, cartilage does not regenerate and instead undergoes progressive degeneration and degradation.

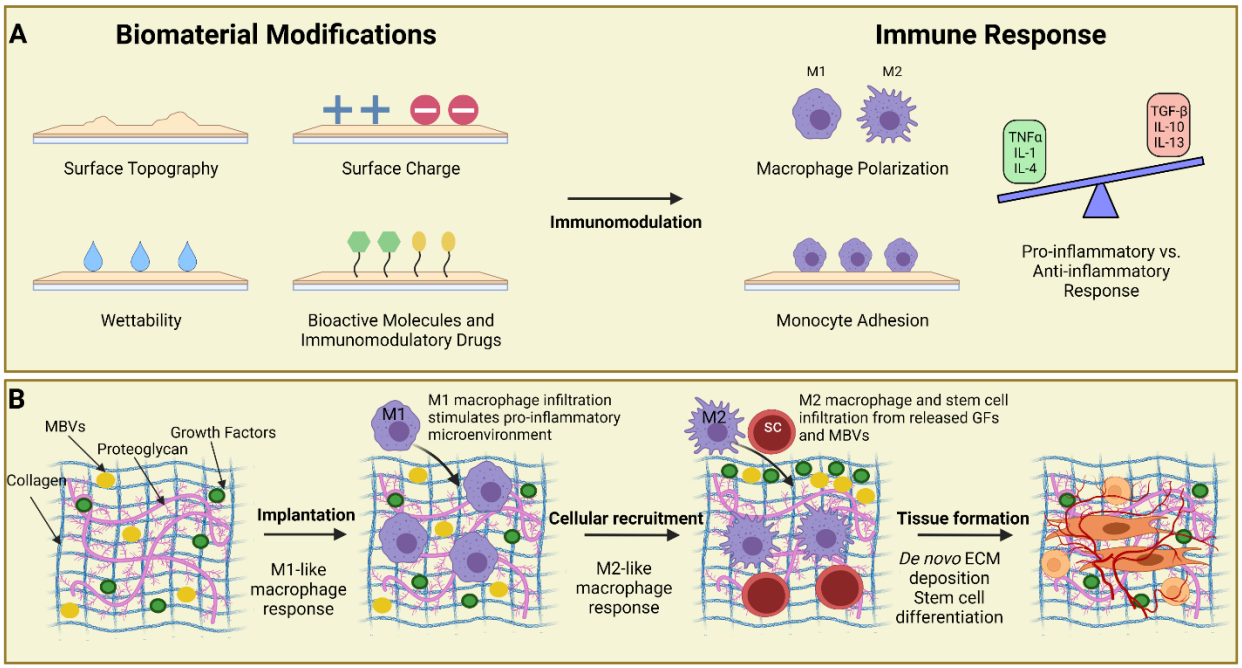

Fig. 2. Immunomodulatory biomaterials for musculoskeletal regeneration. (**A**) Multiple biomaterial modifications including changes to surface topography, surface charge, wettability, and incorporation of bioactive molecules and immunomodulatory drugs can be used to regulate immune-mediated regenerative responses to tissue damage. (**B**) Decellularized extracellular matrices (dECMs) retain multiple biophysical cues which upon implantation stimulate immune cell infiltration and a pro-inflammatory response. Subsequent degradation of the implanted dECM induces release of growth factors and matrix-bound nanovesicles (MBVs) that promote immune cell conversion to an M2 phenotype and stimulate neighboring stem cell recruitment and, ultimately, regeneration via de novo tissue formation.

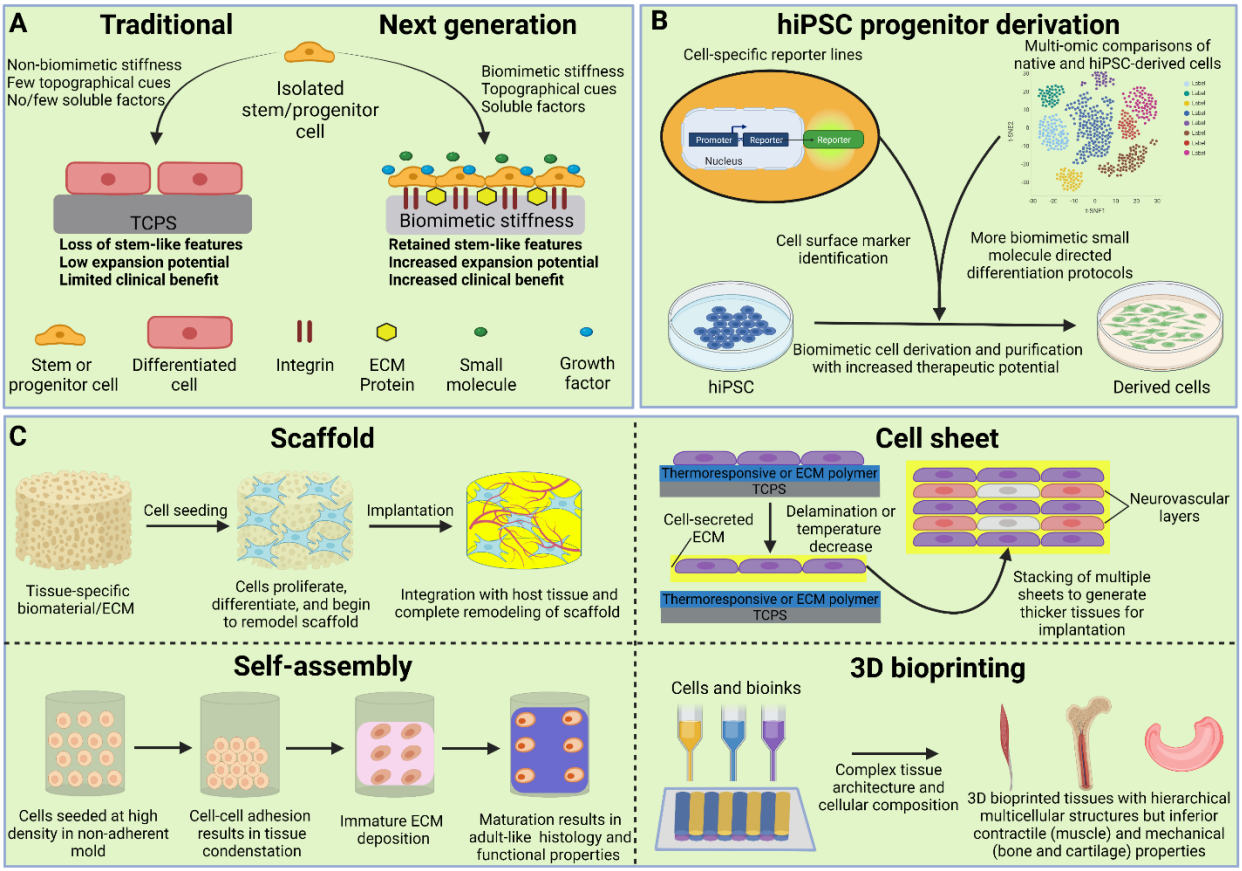

Fig. 3. Bioengineering approaches for cell-based musculoskeletal therapies. (**A**) Traditional cell culture platforms using tissue culture plastic (TCPS) poorly retain stem cell characteristics. Next generation culture platforms retain stem cell characteristics and facilitate cell expansion by better replicating the stem cell niche microenvironment. (**B**) Next generation single-cell sequencing and CRISPR-edited reporter lines allow development of more efficient differentiation protocols for derivation of biomimetic musculoskeletal progenitor cells from hiPSCs. (**C**) Tissue-engineering methods allow in vitro fabrication of functional three-dimensional tissues using: porous scaffolds that initially provide structural and mechanical support to seeded cells and are subsequently remodeled in vitro and in vivo; Cell sheets that are detached from extracellular matrix (ECM)- or thermoresponsive polymer-coated dishes and subsequently stacked; Self-assembly of highly dense cell condensates that initially secrete an immature ECM, followed by cell and matrix

maturation and acquisition of native-like mechanical properties; and 3D bioprinting of cells and bioinks to recreate complex tissue architecture and cell composition, which, however, does not lead to native tissue functionality.

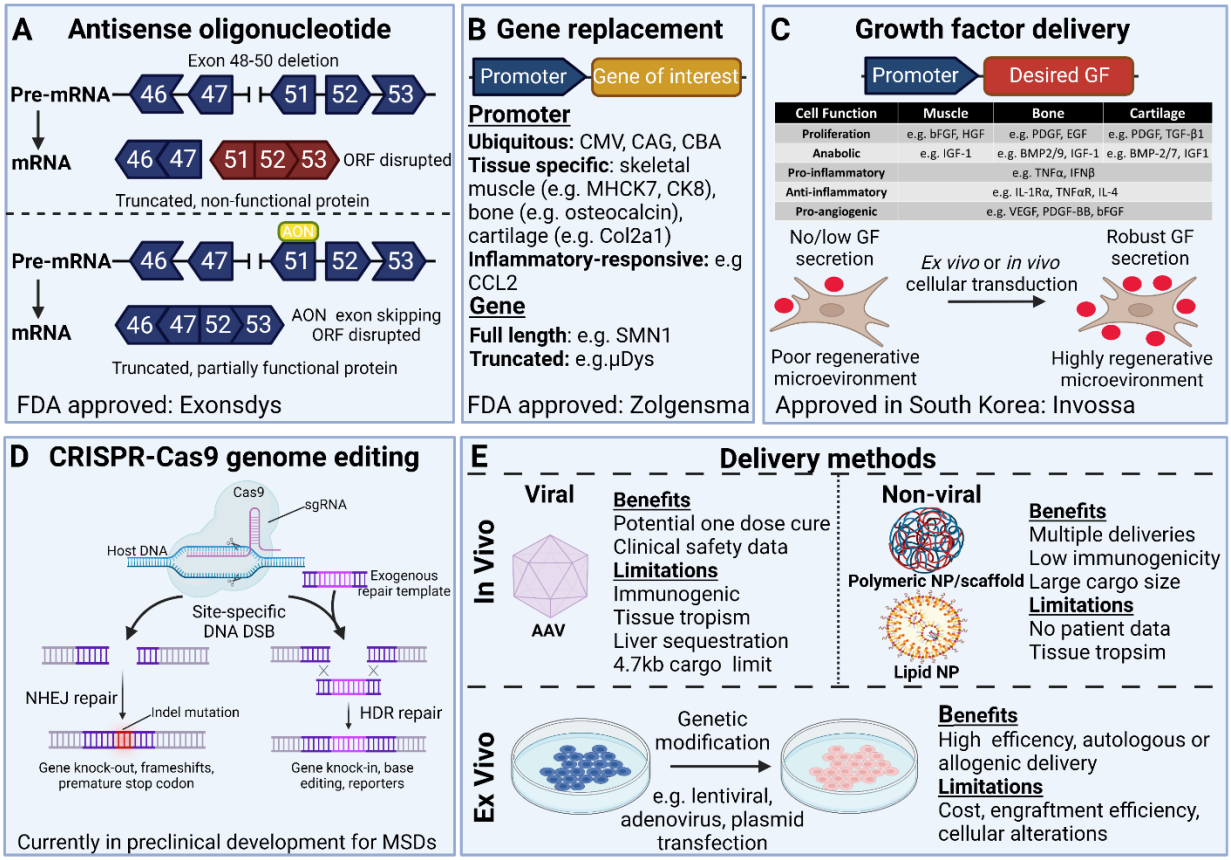

Fig. 4. Bioengineering approaches for gene-based musculoskeletal therapies. (**A**) Antisense oligonucleotides mask exons from splicing machinery and restore functional gene expression. (**B**) Gene replacement via use of ubiquitous, tissue-specific, or inflammatory-responsive promoters controls the expression of full-length or modified versions of the gene of interest. (**C**) Growth factor secretion by ex vivo or in vivo transduced cells creates a pro-regenerative microenvironment at the injury site. (**D**) CRISPR-Cas9 editing induces double-stranded breaks (DSBs) and gene knock-out by nonhomologous end joining (NHEJ). Alternatively, homology-directed repair (HDR) with inclusion of a DNA template allows for gene knock-in. (**E**) Systemic gene delivery is

812 accomplished by AAV vector or non-viral polymeric or lipid nanoparticle (NP) systems.
813 Alternatively, ex vivo gene modifications are performed by transduction or transfection of
814 autologous or allogeneic cells prior to transplantation.

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1. A. Cieza, K. Causey, K. Kamenov, S. W. Hanson, S. Chatterji, T. Vos, Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* **396**, 2006-2017 (2021).
2. M. P. Nikolova, M. S. Chavali, Recent advances in biomaterials for 3D scaffolds: A review. *Bioact Mater* **4**, 271-292 (2019).
3. J. Frobel, H. Hemeda, M. Lenz, G. Abagnale, S. Joussen, B. Denecke, T. Saric, M. Zenke, W. Wagner, Epigenetic rejuvenation of mesenchymal stromal cells derived from induced pluripotent stem cells. *Stem Cell Reports* **3**, 414-422 (2014).
4. H. Zhu, T. Kimura, S. Swami, J. Y. Wu, Pluripotent stem cells as a source of osteoblasts for bone tissue regeneration. *Biomaterials* **196**, 31-45 (2019).
5. H. Xi, J. Langerman, S. Sabri, P. Chien, C. S. Young, S. Younesi, M. Hicks, K. Gonzalez, W. Fujiwara, J. Marzi, S. Liebscher, M. Spencer, B. Van Handel, D. Evseenko, K. Schenke-Layland, K. Plath, A. D. Pyle, A Human Skeletal Muscle Atlas Identifies the Trajectories of Stem and Progenitor Cells across Development and from Human Pluripotent Stem Cells. *Cell Stem Cell* **27**, 158-176 e110 (2020).
6. R. Novak, M. Ingram, S. Marquez, D. Das, A. Delahanty, A. Herland, B. M. Maoz, S. S. F. Jeanty, M. R. Somayaji, M. Burt, E. Calamari, A. Chalkiadaki, A. Cho, Y. Choe, D. B. Chou, M. Cronce, S. Dauth, T. Divic, J. Fernandez-Alcon, T. Ferrante, J. Ferrier, E. A. FitzGerald, R. Fleming, S. Jalili-Firoozinezhad, T. Grevesse, J. A. Goss, T. Hamkins-Indik, O. Henry, C. Hinojosa, T. Huffstater, K. J. Jang, V. Kujala, L. Leng, R. Mannix, Y. Milton, J. Nawroth, B. A. Nestor, C. F. Ng, B. O'Connor, T. E. Park, H. Sanchez, J. Sliz, A. Sontheimer-Phelps, B. Swenor, G. Thompson, 2nd, G. J. Touloumes, Z. Tranchemontagne, N. Wen, M. Yadid, A. Bahinski, G. A. Hamilton, D. Levner, O. Levy, A. Przekwas, R. Prantil-Baun, K. K. Parker, D. E. Ingber, Robotic fluidic coupling and interrogation of multiple vascularized organ chips. *Nat Biomed Eng* **4**, 407-420 (2020).
7. J. Dziki, S. Badylak, M. Yabroudi, B. Sicari, F. Ambrosio, K. Stearns, N. Turner, A. Wyse, M. L. Boninger, E. H. P. Brown, J. P. Rubin, An acellular biologic scaffold treatment for volumetric muscle loss: results of a 13-patient cohort study. *NPJ Regen Med* **1**, 16008 (2016).
8. J. R. Mendell, S. A. Al-Zaidy, K. J. Lehman, M. McColly, L. P. Lowes, L. N. Alfano, N. F. Reash, M. A. Iammarino, K. R. Church, A. Kleyn, M. N. Meriggioli, R. Shell, Five-Year Extension Results of the Phase 1 START Trial of Onasemnogene Abeparvovec in Spinal Muscular Atrophy. *JAMA Neurol* **78**, 834-841 (2021).
9. F. A. Ran, L. Cong, W. X. Yan, D. A. Scott, J. S. Gootenberg, A. J. Kriz, B. Zetsche, O. Shalem, X. B. Wu, K. S. Makarova, E. V. Koonin, P. A. Sharp, F. Zhang, In vivo genome editing using Staphylococcus aureus Cas9. *Nature* **520**, 186-U198 (2015).
10. B. P. Kleinstiver, S. Q. Tsai, M. S. Prew, N. T. Nguyen, M. M. Welch, J. M. Lopez, Z. R. McCaw, M. J. Aryee, J. K. Joung, Genome-wide specificities of CRISPR-Cas Cpf1 nucleases in human cells. *Nature Biotechnology* **34**, 869-+ (2016).
11. A. Iwasaki, R. Medzhitov, Control of adaptive immunity by the innate immune system. *Nat Immunol* **16**, 343-353 (2015).
12. J. G. Tidball, Regulation of muscle growth and regeneration by the immune system. *Nat Rev Immunol* **17**, 165-178 (2017).
13. F. Relaix, M. Bencze, M. J. Borok, A. Der Vartanian, F. Gattazzo, D. Mademtzoglou, S. Perez-Diaz, A. Prola, P. C. Reyes-Fernandez, A. Rotini, t. Taglietti, Perspectives on skeletal muscle stem cells. *Nat Commun* **12**, 692 (2021).
14. J. E. Heredia, L. Mukundan, F. M. Chen, A. A. Mueller, R. C. Deo, R. M. Locksley, T. A. Rando, A. Chawla, Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* **153**, 376-388 (2013).
15. A. W. Joe, L. Yi, A. Natarajan, F. Le Grand, L. So, J. Wang, M. A. Rudnicki, F. M. Rossi, Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nat Cell Biol* **12**, 153-163 (2010).
16. A. Uezumi, S. Fukada, N. Yamamoto, S. Takeda, K. Tsuchida, Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. *Nat Cell Biol* **12**, 143-152 (2010).
17. D. R. Lemos, F. Babaeijandaghi, M. Low, C. K. Chang, S. T. Lee, D. Fiore, R. H. Zhang, A. Natarajan, S. A. Nedospasov, F. M. Rossi, Nilotinib reduces muscle fibrosis in chronic muscle injury by promoting TNF-mediated apoptosis of fibro/adipogenic progenitors. *Nat Med* **21**, 786-794 (2015).

18. G. Giuliani, M. Rosina, A. Reggio, Signaling pathways regulating the fate of fibro/adipogenic progenitors (FAPs) in skeletal muscle regeneration and disease. *FEBS J*, (2021).
19. J. M. Kastenschmidt, G. Coulis, P. K. Farahat, P. Pham, R. Rios, T. T. Cristal, A. H. Mannaa, R. E. Ayer, R. Yahia, A. A. Deshpande, B. S. Hughes, A. K. Savage, C. R. Giesige, S. Q. Harper, R. M. Locksley, T. Mozaffar, S. A. Villalta, A stromal progenitor and ILC2 niche promotes muscle eosinophilia and fibrosis-associated gene expression. *Cell Rep* **35**, 108997 (2021).
20. P. J. Muire, L. H. Mangum, J. C. Wenke, Time Course of Immune Response and Immunomodulation During Normal and Delayed Healing of Musculoskeletal Wounds. *Front Immunol* **11**, 1056 (2020).
21. P. Andrzejowski, P. V. Giannoudis, The 'diamond concept' for long bone non-union management. *J Orthop Traumatol* **20**, 21 (2019).
22. G. Yang, B. B. Rothrauff, R. S. Tuan, Tendon and ligament regeneration and repair: clinical relevance and developmental paradigm. *Birth Defects Res C Embryo Today* **99**, 203-222 (2013).
23. H. Hu, W. Liu, C. Sun, Q. Wang, W. Yang, Z. Zhang, Z. Xia, Z. Shao, B. Wang, Endogenous Repair and Regeneration of Injured Articular Cartilage: A Challenging but Promising Therapeutic Strategy. *Aging Dis* **12**, 886-901 (2021).
24. S. A. Han, S. Lee, S. C. Seong, M. C. Lee, Effects of CD14 macrophages and proinflammatory cytokines on chondrogenesis in osteoarthritic synovium-derived stem cells. *Tissue Eng Part A* **20**, 2680-2691 (2014).
25. Y. Henrotin, B. Kurz, T. Aigner, Oxygen and reactive oxygen species in cartilage degradation: friends or foes? *Osteoarthritis Cartilage* **13**, 643-654 (2005).
26. V. M. Quarch, E. Enderle, J. Lotz, K. H. Frosch, Fate of large donor site defects in osteochondral transfer procedures in the knee joint with and without TruFit plugs. *Arch Orthop Trauma Surg* **134**, 657-666 (2014).
27. E. D. Bonnevie, D. Galesso, C. Secchieri, I. Cohen, L. J. Bonassar, Elastoviscous Transitions of Articular Cartilage Reveal a Mechanism of Synergy between Lubricin and Hyaluronic Acid. *PLoS One* **10**, e0143415 (2015).
28. A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677-689 (2006).
29. P. Viswanathan, M. G. Ondeck, S. Chirasatitsin, K. Ngamkham, G. C. Reilly, A. J. Engler, G. Battaglia, 3D surface topology guides stem cell adhesion and differentiation. *Biomaterials* **52**, 140-147 (2015).
30. X. Zhang, D. Wang, K. K. Mak, R. S. Tuan, D. F. E. Ker, Engineering Musculoskeletal Grafts for Multi-Tissue Unit Repair: Lessons From Developmental Biology and Wound Healing. *Front Physiol* **12**, 691954 (2021).
31. A. Boffa, L. Solaro, A. Poggi, L. Andriolo, D. Reale, A. Di Martino, Multi-layer cell-free scaffolds for osteochondral defects of the knee: a systematic review and meta-analysis of clinical evidence. *J Exp Orthop* **8**, 56 (2021).
32. N. Huettner, T. R. Dargaville, A. Forget, Discovering Cell-Adhesion Peptides in Tissue Engineering: Beyond RGD. *Trends Biotechnol* **36**, 372-383 (2018).
33. M. Quarta, J. O. Brett, R. DiMarco, A. De Morree, S. C. Boutet, R. Chacon, M. C. Gibbons, V. A. Garcia, J. Su, J. B. Shrager, S. Heilshorn, T. A. Rando, An artificial niche preserves the quiescence of muscle stem cells and enhances their therapeutic efficacy. *Nat Biotechnol* **34**, 752-759 (2016).
34. A. Di Benedetto, G. Brunetti, F. Posa, A. Ballini, F. R. Grassi, G. Colaianni, S. Colucci, E. Rossi, E. A. Cavalcanti-Adam, L. Lo Muzio, M. Grano, G. Mori, Osteogenic differentiation of mesenchymal stem cells from dental bud: Role of integrins and cadherins. *Stem Cell Res* **15**, 618-628 (2015).
35. S. J. P. Callens, R. J. C. Uyttendaele, L. E. Fratila-Apachitei, A. A. Zadpoor, Substrate curvature as a cue to guide spatiotemporal cell and tissue organization. *Biomaterials* **232**, 119739 (2020).
36. H. Seong, S. G. Higgins, J. Penders, J. P. K. Armstrong, S. W. Crowder, A. C. Moore, J. E. Sero, M. Becce, M. M. Stevens, Size-Tunable Nanoneedle Arrays for Influencing Stem Cell Morphology, Gene Expression, and Nuclear Membrane Curvature. *ACS Nano* **14**, 5371-5381 (2020).
37. S. Tarafder, S. Bose, Polycaprolactone-coated 3D printed tricalcium phosphate scaffolds for bone tissue engineering: in vitro alendronate release behavior and local delivery effect on in vivo osteogenesis. *ACS Appl Mater Interfaces* **6**, 9955-9965 (2014).
38. L. Huleihel, G. S. Hussey, J. D. Naranjo, L. Zhang, J. L. Dziki, N. J. Turner, D. B. Stolz, S. F. Badylak, Matrix-bound nanovesicles within ECM bioscaffolds. *Science Advances* **2**, (2016).
39. V. Z. Beachley, M. T. Wolf, K. Sadtler, S. S. Manda, H. Jacobs, M. R. Blatchley, J. S. Bader, A. Pandey, D. Pardoll, J. H. Elisseeff, Tissue matrix arrays for high-throughput screening and systems analysis of cell function. *Nat Methods* **12**, 1197-+ (2015).

40. G. Chen, C. Dong, L. Yang, Y. Lv, 3D Scaffolds with Different Stiffness but the Same Microstructure for Bone Tissue Engineering. *ACS Appl Mater Interfaces* **7**, 15790-15802 (2015).
41. G. S. Hussey, J. L. Dziki, S. F. Badylak, Extracellular matrix-based materials for regenerative medicine. *Nat Rev Mater* **3**, 159-173 (2018).
42. S. F. Badylak, J. L. Dziki, B. M. Sicari, F. Ambrosio, M. L. Boninger, Mechanisms by which acellular biologic scaffolds promote functional skeletal muscle restoration. *Biomaterials* **103**, 128-136 (2016).
43. B. M. Sicari, J. P. Rubin, C. L. Dearth, M. T. Wolf, F. Ambrosio, M. Boninger, N. J. Turner, D. J. Weber, T. W. Simpson, A. Wyse, E. H. Brown, J. L. Dziki, L. E. Fisher, S. Brown, S. F. Badylak, An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. *Sci Transl Med* **6**, 234ra258 (2014).
44. T. D. Henry, B. H. Annex, G. R. McKendall, M. A. Azrin, J. J. Lopez, F. J. Giordano, P. K. Shah, J. T. Willerson, R. L. Benza, D. S. Berman, C. M. Gibson, A. Bajamonde, A. C. Rundle, J. Fine, E. R. McCluskey, V. Investigators, The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. *Circulation* **107**, 1359-1365 (2003).
45. Y. M. Ju, A. Atala, J. J. Yoo, S. J. Lee, In situ regeneration of skeletal muscle tissue through host cell recruitment. *Acta Biomater* **10**, 4332-4339 (2014).
46. J. M. Grasman, D. M. Do, R. L. Page, G. D. Pins, Rapid release of growth factors regenerates force output in volumetric muscle loss injuries. *Biomaterials* **72**, 49-60 (2015).
47. S. M. Buchanan, F. D. Price, A. Castiglioni, A. W. Gee, J. Schneider, M. N. Matyas, M. Hayhurst, M. Tabebordbar, A. J. Wagers, L. L. Rubin, Pro-myogenic small molecules revealed by a chemical screen on primary muscle stem cells. *Skeletal muscle* **10**, 28 (2020).
48. J. Fang, J. Sia, J. Soto, P. Wang, L. K. Li, Y. Y. Hsueh, R. Sun, K. F. Faull, J. G. Tidball, S. Li, Skeletal muscle regeneration via the chemical induction and expansion of myogenic stem cells in situ or in vitro. *Nat Biomed Eng* **5**, 864-879 (2021).
49. A. W. James, G. LaChaud, J. Shen, G. Asatrian, V. Nguyen, X. Zhang, K. Ting, C. Soo, A Review of the Clinical Side Effects of Bone Morphogenetic Protein-2. *Tissue Eng Part B Rev* **22**, 284-297 (2016).
50. T. M. De Witte, L. E. Fratila-Apachitei, A. A. Zadpoor, N. A. Peppas, Bone tissue engineering via growth factor delivery: from scaffolds to complex matrices. *Regen Biomater* **5**, 197-211 (2018).
51. T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney, Polymeric system for dual growth factor delivery. *Nat Biotechnol* **19**, 1029-1034 (2001).
52. T. M. Raimondo, H. Li, B. J. Kwee, S. Kinsley, E. Budina, E. M. Anderson, E. J. Doherty, S. G. Talbot, D. J. Mooney, Combined delivery of VEGF and IGF-1 promotes functional innervation in mice and improves muscle transplantation in rabbits. *Biomaterials* **216**, 119246 (2019).
53. R. Subbiah, M. A. Ruehle, B. S. Klosterhoff, A. S. P. Lin, M. H. Hettiaratchi, N. J. Willett, L. E. Bertassoni, A. J. Garcia, R. E. Guldberg, Triple growth factor delivery promotes functional bone regeneration following composite musculoskeletal trauma. *Acta Biomater* **127**, 180-192 (2021).
54. L. A. Fortier, J. U. Barker, E. J. Strauss, T. M. McCarrel, B. J. Cole, The role of growth factors in cartilage repair. *Clin Orthop Relat Res* **469**, 2706-2715 (2011).
55. L. S. Lohmander, S. Hellot, D. Dreher, E. F. Krantz, D. S. Kruger, A. Guermazi, F. Eckstein, Intraarticular sprifermin (recombinant human fibroblast growth factor 18) in knee osteoarthritis: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheumatol* **66**, 1820-1831 (2014).
56. A. G. Bajpayee, C. R. Wong, M. G. Bawendi, E. H. Frank, A. J. Grodzinsky, Avidin as a model for charge driven transport into cartilage and drug delivery for treating early stage post-traumatic osteoarthritis. *Biomaterials* **35**, 538-549 (2014).
57. B. C. Geiger, S. Wang, R. F. Padera, A. J. Grodzinsky, P. T. Hammond, Cartilage-penetrating nanocarriers improve delivery and efficacy of growth factor treatment of osteoarthritis. *Science Translational Medicine* **10**, (2018).
58. A. C. Pochini, E. Antonioli, D. Z. Bucci, L. R. Sardinha, C. V. Andreoli, M. Ferretti, B. Ejnisman, A. C. Goldberg, M. Cohen, Analysis of cytokine profile and growth factors in platelet-rich plasma obtained by open systems and commercial columns. *Einstein (Sao Paulo)* **14**, 391-397 (2016).
59. A. Grassi, F. Napoli, I. Romandini, K. Samuelsson, S. Zaffagnini, C. Candrian, G. Filardo, Is Platelet-Rich Plasma (PRP) Effective in the Treatment of Acute Muscle Injuries? A Systematic Review and Meta-Analysis. *Sports Med* **48**, 971-989 (2018).
60. A. Pocaterra, S. Caruso, S. Bernardi, L. Scagnoli, M. A. Continenza, R. Gatto, Effectiveness of platelet-rich plasma as an adjunctive material to bone graft: a systematic review and meta-analysis of randomized controlled clinical trials. *Int J Oral Maxillofac Surg* **45**, 1027-1034 (2016).

61. G. Filardo, D. Previtali, F. Napoli, C. Candrian, S. Zaffagnini, A. Grassi, PRP Injections for the Treatment of Knee Osteoarthritis: A Meta-Analysis of Randomized Controlled Trials. *Cartilage*, 1947603520931170 (2020).
62. C. R. Chu, S. Rodeo, N. Bhutani, L. R. Goodrich, J. Huard, J. Irrgang, R. F. LaPrade, C. Lattermann, Y. Lu, B. Mandelbaum, J. Mao, L. McIntyre, A. Mishra, G. F. Muschler, N. S. Piuzzi, H. Potter, K. Spindler, J. M. Tokish, R. Tuan, K. Zaslav, W. Maloney, Optimizing Clinical Use of Biologics in Orthopaedic Surgery: Consensus Recommendations From the 2018 AAOS/NIH U-13 Conference. *J Am Acad Orthop Surg* **27**, e50-e63 (2019).
63. F. S. M. Yamaguchi, S. Shams, E. A. Silva, R. S. Stilhano, PRP and BMAC for Musculoskeletal Conditions via Biomaterial Carriers. *Int J Mol Sci* **20**, (2019).
64. M. L. Louis, J. Magalon, E. Jouve, C. E. Bornet, J. C. Mattei, C. Chagnaud, A. Rochwerger, J. Veran, F. Sabatier, Growth Factors Levels Determine Efficacy of Platelets Rich Plasma Injection in Knee Osteoarthritis: A Randomized Double Blind Noninferiority Trial Compared With Viscosupplementation. *Arthroscopy-the Journal of Arthroscopic and Related Surgery* **34**, 1530-+ (2018).
65. F. Kimura, H. Shimizu, H. Yoshidome, M. Ohtsuka, M. Miyazaki, Immunosuppression following surgical and traumatic injury. *Surg Today* **40**, 793-808 (2010).
66. E. C. Smith, L. S. Conklin, E. P. Hoffman, P. R. Clemens, J. K. Mah, R. S. Finkel, M. Guglieri, M. Tulinius, Y. Nevo, M. M. Ryan, R. Webster, D. Castro, N. L. Kuntz, L. Kerchner, L. P. Morgenroth, A. Arrieta, M. Shimony, M. Jaros, P. Shale, H. Gordish-Dressman, L. Hagerty, U. J. Dang, J. M. Damsker, B. D. Schwartz, L. J. Mengle-Gaw, C. M. McDonald, V. B. P. Cinrg, D. Investigators, Efficacy and safety of vamorolone in Duchenne muscular dystrophy: An 18-month interim analysis of a non-randomized open-label extension study. *PLoS Med* **17**, e1003222 (2020).
67. A. Cheng, C. E. Vantucci, L. Krishnan, M. A. Ruehle, T. Kotanchek, L. B. Wood, K. Roy, R. E. Guldberg, Early systemic immune biomarkers predict bone regeneration after trauma. *Proc Natl Acad Sci U S A* **118**, (2021).
68. S. A. Eming, T. A. Wynn, P. Martin, Inflammation and metabolism in tissue repair and regeneration. *Science* **356**, 1026-1030 (2017).
69. M. L. Novak, E. M. Weinheimer-Haus, T. J. Koh, Macrophage activation and skeletal muscle healing following traumatic injury. *J Pathol* **232**, 344-355 (2014).
70. V. Rybalko, P. L. Hsieh, M. Merscham-Banda, L. J. Suggs, R. P. Farrar, The Development of Macrophage-Mediated Cell Therapy to Improve Skeletal Muscle Function after Injury. *PLoS One* **10**, e0145550 (2015).
71. L. Martins, C. C. Gallo, T. S. B. Honda, P. T. Alves, R. S. Stilhano, D. S. Rosa, T. J. Koh, S. W. Han, Skeletal muscle healing by M1-like macrophages produced by transient expression of exogenous GM-CSF. *Stem Cell Res Ther* **11**, 473 (2020).
72. B. N. Brown, R. Londono, S. Tottey, L. Zhang, K. A. Kukla, M. T. Wolf, K. A. Daly, J. E. Reing, S. F. Badylak, Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials. *Acta Biomater* **8**, 978-987 (2012).
73. M. A. Dobrovolskaia, S. E. McNeil, Immunological properties of engineered nanomaterials. *Nat Nanotechnol* **2**, 469-478 (2007).
74. W. G. Brodbeck, Y. Nakayama, T. Matsuda, E. Colton, N. P. Ziats, J. M. Anderson, Biomaterial surface chemistry dictates adherent monocyte/macrophage cytokine expression in vitro. *Cytokine* **18**, 311-319 (2002).
75. E. M. Sussman, M. C. Halpin, J. Muster, R. T. Moon, B. D. Ratner, Porous implants modulate healing and induce shifts in local macrophage polarization in the foreign body reaction. *Ann Biomed Eng* **42**, 1508-1516 (2014).
76. G. Han, Q. Chen, F. Liu, Z. Cui, H. Shao, F. Liu, A. Ma, J. Liao, B. Guo, Y. Guo, F. Wang, P. Ling, X. Mei, Low molecular weight xanthan gum for treating osteoarthritis. *Carbohydr Polym* **164**, 386-395 (2017).
77. M. Dai, B. Sui, Y. Xue, X. Liu, J. Sun, Cartilage repair in degenerative osteoarthritis mediated by squid type II collagen via immunomodulating activation of M2 macrophages, inhibiting apoptosis and hypertrophy of chondrocytes. *Biomaterials* **180**, 91-103 (2018).
78. T. M. Raimondo, D. J. Mooney, Functional muscle recovery with nanoparticle-directed M2 macrophage polarization in mice. *Proc Natl Acad Sci U S A* **115**, 10648-10653 (2018).
79. T. M. Raimondo, D. J. Mooney, Anti-inflammatory nanoparticles significantly improve muscle function in a murine model of advanced muscular dystrophy. *Sci Adv* **7**, (2021).
80. T. Partridge, Myoblast transplantation. *Neuromuscul Disord* **12 Suppl 1**, S3-6 (2002).

81. D. Montarras, J. Morgan, C. Collins, F. Relaix, S. Zaffran, A. Cumano, T. Partridge, M. Buckingham, Direct isolation of satellite cells for skeletal muscle regeneration. *Science* **309**, 2064-2067 (2005).
82. G. W. Charville, T. H. Cheung, B. Yoo, P. J. Santos, G. K. Lee, J. B. Shrager, T. A. Rando, Ex Vivo Expansion and In Vivo Self-Renewal of Human Muscle Stem Cells. *Stem Cell Reports* **5**, 621-632 (2015).
83. B. D. Cosgrove, P. M. Gilbert, E. Porpiglia, F. Mourkioti, S. P. Lee, S. Y. Corbel, M. E. Llewellyn, S. L. Delp, H. M. Blau, Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med* **20**, 255-264 (2014).
84. P. M. Gilbert, K. L. Havenstrite, K. E. Magnusson, A. Sacco, N. A. Leonardi, P. Kraft, N. K. Nguyen, S. Thrun, M. P. Lutolf, H. M. Blau, Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* **329**, 1078-1081 (2010).
85. M. Brittberg, A. Lindahl, A. Nilsson, C. Ohlsson, O. Isaksson, L. Peterson, Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* **331**, 889-895 (1994).
86. E. M. Darling, K. A. Athanasiou, Rapid phenotypic changes in passaged articular chondrocyte subpopulations. *J Orthop Res* **23**, 425-432 (2005).
87. H. Kwon, W. E. Brown, S. A. O'Leary, J. C. Hu, K. A. Athanasiou, Rejuvenation of extensively passaged human chondrocytes to engineer functional articular cartilage. *Biofabrication*, (2021).
88. E. Gomez-Barrena, P. Rosset, D. Lozano, J. Stanovici, C. Ermthaller, F. Gerbhard, Bone fracture healing: cell therapy in delayed unions and nonunions. *Bone* **70**, 93-101 (2015).
89. O. Levy, R. Kuai, E. M. J. Siren, D. Bhere, Y. Milton, N. Nissar, M. De Biasio, M. Heinelt, B. Reeve, R. Abdi, M. Alturki, M. Fallatah, A. Almalik, A. H. Alhasan, K. Shah, J. M. Karp, Shattering barriers toward clinically meaningful MSC therapies. *Sci Adv* **6**, eaba6884 (2020).
90. A. R. Killaars, J. C. Grim, C. J. Walker, E. A. Hushka, T. E. Brown, K. S. Anseth, Extended Exposure to Stiff Microenvironments Leads to Persistent Chromatin Remodeling in Human Mesenchymal Stem Cells. *Adv Sci (Weinh)* **6**, 1801483 (2019).
91. J. Kim, A. Magli, S. S. K. Chan, V. K. P. Oliveira, J. Wu, R. Darabi, M. Kyba, R. C. R. Perlingeiro, Expansion and Purification Are Critical for the Therapeutic Application of Pluripotent Stem Cell-Derived Myogenic Progenitors. *Stem Cell Reports* **9**, 12-22 (2017).
92. A. Magli, T. Incitti, J. Kiley, S. A. Swanson, R. Darabi, F. Rinaldi, S. Selvaraj, A. Yamamoto, J. Tolar, C. Yuan, R. Stewart, J. A. Thomson, R. C. R. Perlingeiro, PAX7 Targets, CD54, Integrin alpha9beta1, and SDC2, Allow Isolation of Human ESC/iPSC-Derived Myogenic Progenitors. *Cell Rep* **19**, 2867-2877 (2017).
93. T. Incitti, A. Magli, R. Darabi, C. Yuan, K. Lin, R. W. Arpke, K. Azzag, A. Yamamoto, R. Stewart, J. A. Thomson, M. Kyba, R. C. R. Perlingeiro, Pluripotent stem cell-derived myogenic progenitors remodel their molecular signature upon in vivo engraftment. *Proc Natl Acad Sci U S A*, (2019).
94. P. Jungbluth, L. S. Spitzhorn, J. Grassmann, S. Tanner, D. Latz, M. S. Rahman, M. Bohndorf, W. Wruck, M. Sager, V. Grotheer, P. Kropil, M. Hakimi, J. Windolf, J. Schneppendahl, J. Adjaye, Human iPSC-derived iMSCs improve bone regeneration in mini-pigs. *Bone Res* **7**, 32 (2019).
95. O. H. Jeon, L. M. Panicker, Q. Lu, J. J. Chae, R. A. Feldman, J. H. Elisseeff, Human iPSC-derived osteoblasts and osteoclasts together promote bone regeneration in 3D biomaterials. *Sci Rep* **6**, 26761 (2016).
96. D. Zujur, K. Kanke, S. Onodera, S. Tani, J. Lai, T. Azuma, X. Xin, A. C. Lichtler, D. W. Rowe, T. Saito, S. Tanaka, H. Masaki, H. Nakauchi, U. I. Chung, H. Hojo, S. Ohba, Stepwise strategy for generating osteoblasts from human pluripotent stem cells under fully defined xeno-free conditions with small-molecule inducers. *Regen Ther* **14**, 19-31 (2020).
97. A. Dicks, C. L. Wu, N. Steward, S. S. Adkar, C. A. Gersbach, F. Guilak, Prospective isolation of chondroprogenitors from human iPSCs based on cell surface markers identified using a CRISPR-Cas9-generated reporter. *Stem Cell Research & Therapy* **11**, (2020).
98. C. L. Wu, A. Dicks, N. Steward, R. Tang, D. B. Katz, Y. R. Choi, F. Guilak, Single cell transcriptomic analysis of human pluripotent stem cell chondrogenesis. *Nat Commun* **12**, 362 (2021).
99. H. Okano, M. Nakamura, K. Yoshida, Y. Okada, O. Tsuji, S. Nori, E. Ikeda, S. Yamanaka, K. Miura, Steps toward safe cell therapy using induced pluripotent stem cells. *Circulation research* **112**, 523-533 (2013).
100. I. R. de Luzy, K. C. L. Law, N. Moriarty, C. P. J. Hunt, J. C. Durnall, L. H. Thompson, A. Nagy, C. L. Parish, Human stem cells harboring a suicide gene improve the safety and standardisation of neural transplants in Parkinsonian rats. *Nat Commun* **12**, 3275 (2021).
101. Y. Torrente, M. Belicchi, C. Marchesi, G. D'Antona, F. Cogiamanian, F. Pisati, M. Gavina, R. Giordano, R. Tonlorenzi, G. Fagiolari, C. Lamperti, L. Porretti, R. Lopa, M. Sampaolesi, L. Vicentini, N. Grimoldi, F.

Tiberio, V. Songa, P. Baratta, A. Prelle, L. Forzenigo, M. Guglieri, O. Pansarasa, C. Rinaldi, V. Mouly, G. S. Butler-Browne, G. P. Comi, P. Biondetti, M. Moggio, S. M. Gaini, N. Stocchetti, A. Priori, M. G. D'Angelo, A. Turconi, R. Bottinelli, G. Cossu, P. Rebulla, N. Bresolin, Autologous transplantation of muscle-derived CD133+ stem cells in Duchenne muscle patients. *Cell Transplant* **16**, 563-577 (2007).

102. G. Cossu, S. C. Previtali, S. Napolitano, M. P. Cicalese, F. S. Tedesco, F. Nicastro, M. Noviello, U. Roostalu, M. G. Natali Sora, M. Scarlato, M. De Pellegrin, C. Godi, S. Giuliani, F. Ciotti, R. Tonlorenzi, I. Lorenzetti, C. Rivellini, S. Benedetti, R. Gatti, S. Marktel, B. Mazzi, A. Tettamanti, M. Ragazzi, M. A. Imro, G. Marano, A. Ambrosi, R. Fiori, M. P. Sormani, C. Bonini, M. Venturini, L. S. Politi, Y. Torrente, F. Ciceri, Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. *EMBO Mol Med* **7**, 1513-1528 (2015).

103. O. Levy, L. J. Mortensen, G. Boquet, Z. Tong, C. Perrault, B. Benhamou, J. Zhang, T. Stratton, E. Han, H. Safaee, J. Musabeyezu, Z. Yang, M. C. Multon, J. Rothblatt, J. F. Deleuze, C. P. Lin, J. M. Karp, A small-molecule screen for enhanced homing of systemically infused cells. *Cell Rep* **10**, 1261-1268 (2015).

104. M. Shi, J. Li, L. Liao, B. Chen, B. Li, L. Chen, H. Jia, R. C. Zhao, Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice. *Haematologica* **92**, 897-904 (2007).

105. B. Corradetti, F. Taraballi, J. O. Martinez, S. Minardi, N. Basu, G. Bauza, M. Evangelopoulos, S. Powell, C. Corbo, E. Tasciotti, Hyaluronic acid coatings as a simple and efficient approach to improve MSC homing toward the site of inflammation. *Scientific Reports* **7**, (2017).

106. T. Schioppa, B. Uranchimeg, A. Saccani, S. K. Biswas, A. Doni, A. Rapisarda, S. Bernasconi, S. Saccani, M. Nebuloni, L. Vago, A. Mantovani, G. Melillo, A. Sica, Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med* **198**, 1391-1402 (2003).

107. S. Bobis-Wozowicz, K. Miekus, E. Wybieralska, D. Jarocha, A. Zawisz, Z. Madeja, M. Majka, Genetically modified adipose tissue-derived mesenchymal stem cells overexpressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of NOD/SCID mice. *Exp Hematol* **39**, 686-696 e684 (2011).

108. M. Costantini, S. Testa, E. Fornetti, C. Fuoco, C. Sanchez Riera, M. Nie, S. Bernardini, A. Rainer, J. Baldi, C. Zoccali, R. Biagini, L. Castagnoli, L. Vitiello, B. Blaauw, D. Seliktar, W. Swieszkowski, P. Garstecki, S. Takeuchi, G. Cesareni, S. Cannata, C. Gargioli, Biofabricating murine and human myo-substitutes for rapid volumetric muscle loss restoration. *EMBO Mol Med* **13**, e12778 (2021).

109. S. Salehi, S. Ostrovidov, M. Ebrahimi, R. B. Sadeghian, X. Liang, K. Nakajima, H. Bae, T. Fujie, A. Khademhosseini, Development of Flexible Cell-Loaded Ultrathin Ribbons for Minimally Invasive Delivery of Skeletal Muscle Cells. *ACS Biomater Sci Eng* **3**, 579-589 (2017).

110. M. Brittberg, Cell carriers as the next generation of cell therapy for cartilage repair: a review of the matrix-induced autologous chondrocyte implantation procedure. *Am J Sports Med* **38**, 1259-1271 (2010).

111. K. J. Caldwell, S. Gottschalk, A. C. Talleur, Allogeneic CAR Cell Therapy-More Than a Pipe Dream. *Front Immunol* **11**, 618427 (2020).

112. H. Xu, B. Wang, M. Ono, A. Kagita, K. Fujii, N. Sasakawa, T. Ueda, P. Gee, M. Nishikawa, M. Nomura, F. Kitaoka, T. Takahashi, K. Okita, Y. Yoshida, S. Kaneko, A. Hotta, Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility. *Cell Stem Cell* **24**, 566-578 e567 (2019).

113. X. P. Huang, Z. Sun, Y. Miyagi, H. McDonald Kinkaid, L. Zhang, R. D. Weisel, R. K. Li, Differentiation of allogeneic mesenchymal stem cells induces immunogenicity and limits their long-term benefits for myocardial repair. *Circulation* **122**, 2419-2429 (2010).

114. P. F. Lesault, M. Theret, M. Magnan, S. Cuvellier, Y. Niu, R. K. Gherardi, J. P. Tremblay, L. Hittinger, B. Chazaud, Macrophages improve survival, proliferation and migration of engrafted myogenic precursor cells into MDX skeletal muscle. *PLoS One* **7**, e46698 (2012).

115. M. Juhas, N. Abutaleb, J. T. Wang, J. Ye, Z. Shaikh, C. Sriworarat, Y. Qian, N. Bursac, Incorporation of macrophages into engineered skeletal muscle enables enhanced muscle regeneration. *Nat Biomed Eng* **2**, 942-954 (2018).

116. R. G. Dennis, P. E. Kosnik, 2nd, Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. *In Vitro Cell Dev Biol Anim* **36**, 327-335 (2000).

117. E. Nagamori, T. X. Ngo, Y. Takezawa, A. Saito, Y. Sawa, T. Shimizu, T. Okano, M. Taya, M. Kino-oka, Network formation through active migration of human vascular endothelial cells in a multilayered skeletal myoblast sheet. *Biomaterials* **34**, 662-668 (2013).

118. N. E. Ryu, S. H. Lee, H. Park, Spheroid Culture System Methods and Applications for Mesenchymal Stem Cells. *Cells* **8**, (2019).
119. J. K. Lee, J. M. Link, J. C. Y. Hu, K. A. Athanasiou, The Self-Assembling Process and Applications in Tissue Engineering. *Cold Spring Harb Perspect Med* **7**, (2017).
120. A. A. Athens, E. A. Makris, J. C. Hu, Induced collagen cross-links enhance cartilage integration. *PLoS One* **8**, e60719 (2013).
121. J. C. Hu, K. A. Athanasiou, A self-assembling process in articular cartilage tissue engineering. *Tissue Eng* **12**, 969-979 (2006).
122. L. Rao, Y. Qian, A. Khodabukus, T. Ribar, N. Bursac, Engineering human pluripotent stem cells into a functional skeletal muscle tissue. *Nat Commun* **9**, 126 (2018).
123. L. Madden, M. Juhas, W. E. Kraus, G. A. Truskey, N. Bursac, Bioengineered human myobundles mimic clinical responses of skeletal muscle to drugs. *Elife* **4**, e04885 (2015).
124. L. Perry, M. Y. Flugelman, S. Levenberg, Elderly Patient-Derived Endothelial Cells for Vascularization of Engineered Muscle. *Mol Ther* **25**, 935-948 (2017).
125. S. S. Novakova, B. L. Rodriguez, E. E. Vega-Soto, G. P. Nutter, R. E. Armstrong, P. C. D. Macpherson, L. M. Larkin, Repairing Volumetric Muscle Loss in the Ovine Peroneus Tertius Following a 3-Month Recovery. *Tissue Eng Part A* **26**, 837-851 (2020).
126. J. Ng, K. Spiller, J. Bernhard, G. Vunjak-Novakovic, Biomimetic Approaches for Bone Tissue Engineering. *Tissue Eng Part B Rev* **23**, 480-493 (2017).
127. R. Witt, A. Weigand, A. M. Boos, A. Cai, D. Dippold, A. R. Boccaccini, D. W. Schubert, M. Hardt, C. Lange, A. Arkudas, R. E. Horch, J. P. Beier, Mesenchymal stem cells and myoblast differentiation under HGF and IGF-1 stimulation for 3D skeletal muscle tissue engineering. *BMC Cell Biol* **18**, 15 (2017).
128. N. Mohan, N. H. Dormer, K. L. Caldwell, V. H. Key, C. J. Berkland, M. S. Detamore, Continuous gradients of material composition and growth factors for effective regeneration of the osteochondral interface. *Tissue Eng Part A* **17**, 2845-2855 (2011).
129. A. Di Luca, M. Klein-Gunnewiek, J. G. Vancso, C. A. van Blitterswijk, E. M. Benetti, L. Moroni, Covalent Binding of Bone Morphogenetic Protein-2 and Transforming Growth Factor-beta3 to 3D Plotted Scaffolds for Osteochondral Tissue Regeneration. *Biotechnol J* **12**, (2017).
130. A. Khodabukus, L. Madden, N. K. Prabhu, T. R. Koves, C. P. Jackman, D. M. Muoio, N. Bursac, Electrical stimulation increases hypertrophy and metabolic flux in tissue-engineered human skeletal muscle. *Biomaterials* **198**, 259-269 (2019).
131. C. A. Powell, B. L. Smiley, J. Mills, H. H. Vandenburgh, Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am J Physiol Cell Physiol* **283**, C1557-1565 (2002).
132. C. Y. Huang, R. Ogawa, Effect of Hydrostatic Pressure on Bone Regeneration Using Human Mesenchymal Stem Cells. *Tissue Eng Pt A* **18**, 2106-2113 (2012).
133. A. Ravichandran, J. Lim, M. S. K. Chong, F. Wen, Y. Liu, Y. T. Pillay, J. K. Y. Chan, S. H. Teoh, In vitro cyclic compressive loads potentiate early osteogenic events in engineered bone tissue. *J Biomed Mater Res B Appl Biomater* **105**, 2366-2375 (2017).
134. Y. Q. Kang, N. Mochizuki, A. Khademhosseini, J. Fukuda, Y. Z. Yang, Engineering a vascularized collagen-beta-tricalcium phosphate graft using an electrochemical approach. *Acta Biomaterialia* **11**, 449-458 (2015).
135. S. Levenberg, J. Rouwkema, M. Macdonald, E. S. Garfein, D. S. Kohane, D. C. Darland, R. Marini, C. A. van Blitterswijk, R. C. Mulligan, P. A. D'Amore, R. Langer, Engineering vascularized skeletal muscle tissue. *Nat Biotechnol* **23**, 879-884 (2005).
136. D. C. Miller, A. Thapa, K. M. Haberstroh, T. J. Webster, Endothelial and vascular smooth muscle cell function on poly(lactic-co-glycolic acid) with nano-structured surface features. *Biomaterials* **25**, 53-61 (2004).
137. J. Koffler, K. Kaufman-Francis, Y. Shandalov, D. Egozi, D. A. Pavlov, A. Landesberg, S. Levenberg, Improved vascular organization enhances functional integration of engineered skeletal muscle grafts. *Proc Natl Acad Sci U S A* **108**, 14789-14794 (2011).
138. J. H. Kim, I. K. Ko, M. J. Jeon, I. Kim, M. M. Vanschaayk, A. Atala, J. J. Yoo, Pelvic floor muscle function recovery using biofabricated tissue constructs with neuromuscular junctions. *Acta Biomater* **121**, 237-249 (2021).
139. W. Bian, N. Bursac, Soluble miniagrin enhances contractile function of engineered skeletal muscle. *FASEB J* **26**, 955-965 (2012).

140. J. Gilbert-Honick, S. R. Iyer, S. M. Somers, H. Takasuka, R. M. Lovering, K. R. Wagner, H. Q. Mao, W. L. Grayson, Engineering 3D skeletal muscle primed for neuromuscular regeneration following volumetric muscle loss. *Biomaterials* **255**, 120154 (2020).
141. M. Ebrahimi, S. Ostrovidov, S. Salehi, S. B. Kim, H. Bae, A. Khademhosseini, Enhanced skeletal muscle formation on microfluidic spun gelatin methacryloyl (GelMA) fibres using surface patterning and agrin treatment. *J Tissue Eng Regen Med* **12**, 2151-2163 (2018).
142. Y. F. Zhang, J. K. Xu, Y. C. Ruan, M. K. Yu, M. O'Laughlin, H. Wise, D. Chen, L. Tian, D. F. Shi, J. L. Wang, S. H. Chen, J. Q. Feng, D. H. K. Chow, X. H. Xie, L. Z. Zheng, L. Huang, S. Huang, K. Leung, N. Lu, L. Zhao, H. F. Li, D. W. Zhao, X. Guo, K. M. Chan, F. Witte, H. C. Chan, Y. F. Zheng, L. Qin, Implant-derived magnesium induces local neuronal production of CGRP to improve bone-fracture healing in rats. *Nature Medicine* **22**, 1160-1169 (2016).
143. V. Dhawan, I. F. Lytle, D. E. Dow, Y. C. Huang, D. L. Brown, Neurotization improves contractile forces of tissue-engineered skeletal muscle. *Tissue Eng* **13**, 2813-2821 (2007).
144. Y. Wu, D. Jing, H. W. Ouyang, L. Li, M. M. Zhai, Y. Li, L. Bi, G. X. Pei, Pre-implanted Sensory Nerve Could Enhance the Neurotization in Tissue-Engineered Bone Graft. *Tissue Eng Pt A* **21**, 2241-2249 (2015).
145. J. H. Kim, I. Kim, Y. J. Seol, I. K. Ko, J. J. Yoo, A. Atala, S. J. Lee, Neural cell integration into 3D bioprinted skeletal muscle constructs accelerates restoration of muscle function. *Nat Commun* **11**, 1025 (2020).
146. A. M. Adams, K. W. VanDusen, T. Y. Kostrominova, J. P. Mertens, L. M. Larkin, Scaffoldless tissue-engineered nerve conduit promotes peripheral nerve regeneration and functional recovery after tibial nerve injury in rats. *Neural Regen Res* **12**, 1529-1537 (2017).
147. S. Ostrovidov, S. Salehi, M. Costantini, K. Suthiwanich, M. Ebrahimi, R. B. Sadeghian, T. Fujie, X. Shi, S. Cannata, C. Gargioli, A. Tamayol, M. R. Dokmeci, G. Orive, W. Swieszkowski, A. Khademhosseini, 3D Bioprinting in Skeletal Muscle Tissue Engineering. *Small* **15**, e1805530 (2019).
148. H. W. Kang, S. J. Lee, I. K. Ko, C. Kengla, J. J. Yoo, A. Atala, A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol* **34**, 312-319 (2016).
149. A. Mostafavi, M. Samandari, M. Karvar, M. Ghovvati, Y. Endo, I. Sinha, N. Annabi, A. Tamayol, Colloidal multiscale porous adhesive (bio)inks facilitate scaffold integration. *Appl Phys Rev* **8**, 041415 (2021).
150. T. Potyondy, J. A. Uquillas, P. J. Tebon, B. Byambaa, A. Hasan, M. Tavafoghi, H. Mary, G. Aninwene Ii, I. Pountos, A. Khademhosseini, N. Ashammakhi, Recent advances in 3D bioprinting of musculoskeletal tissues. *Biofabrication*, (2020).
151. K. H. Nakayama, C. Alcazar, G. Yang, M. Quarta, P. Paine, L. Doan, A. Davies, T. A. Rando, N. F. Huang, Rehabilitative exercise and spatially patterned nanofibrillar scaffolds enhance vascularization and innervation following volumetric muscle loss. *NPJ Regen Med* **3**, 16 (2018).
152. M. Quarta, M. Cromie, R. Chacon, J. Blonigan, V. Garcia, I. Akimenko, M. Hamer, P. Paine, M. Stok, J. B. Shrager, T. A. Rando, Bioengineered constructs combined with exercise enhance stem cell-mediated treatment of volumetric muscle loss. *Nat Commun* **8**, 15613 (2017).
153. J. Wang, A. Khodabukus, L. Rao, K. Vandusen, N. Abutaleb, N. Bursac, Engineered skeletal muscles for disease modeling and drug discovery. *Biomaterials* **221**, 119416 (2019).
154. A. Mansoorifar, R. Gordon, R. C. Bergan, L. E. Bertassoni, Bone-on-a-Chip: Microfluidic Technologies and Microphysiologic Models of Bone Tissue. *Adv Funct Mater* **31**, (2021).
155. C. A. Paggi, L. M. Teixeira, S. Le Gac, M. Karperien, Joint-on-chip platforms: entering a new era of in vitro models for arthritis. *Nat Rev Rheumatol*, (2022).
156. T. Osaki, S. G. M. Uzel, R. D. Kamm, Microphysiological 3D model of amyotrophic lateral sclerosis (ALS) from human iPS-derived muscle cells and optogenetic motor neurons. *Sci Adv* **4**, eaat5847 (2018).
157. O. F. Vila, M. Chavez, S. P. Ma, K. Yeager, L. V. Zholudeva, J. M. Colon-Mercado, Y. Qu, T. R. Nash, C. Lai, C. M. Feliciano, M. Carter, R. D. Kamm, L. M. Judge, B. R. Conklin, M. E. Ward, T. C. McDevitt, G. Vunjak-Novakovic, Bioengineered optogenetic model of human neuromuscular junction. *Biomaterials* **276**, 121033 (2021).
158. Z. Fralish, E. M. Lotz, T. Chavez, A. Khodabukus, N. Bursac, Neuromuscular Development and Disease: Learning From in vitro and in vivo Models. *Front Cell Dev Biol* **9**, 764732 (2021).
159. P. Occhetta, A. Mainardi, E. Votta, Q. Vallmajo-Martin, M. Ehrbar, I. Martin, A. Barbero, M. Rasponi, Hyperphysiological compression of articular cartilage induces an osteoarthritic phenotype in a cartilage-on-a-chip model. *Nat Biomed Eng* **3**, 545-557 (2019).

160. M. Rothbauer, G. Holl, C. Eilenberger, S. R. A. Kratz, B. Farooq, P. Schuller, I. O. Calvo, R. A. Byrne, B. Meyer, B. Niederreiter, S. Kupcu, F. Sevelda, J. Holinka, O. Hayden, S. F. Tedde, H. P. Kiener, P. Ertl, Monitoring tissue-level remodelling during inflammatory arthritis using a three-dimensional synovium-on-a-chip with non-invasive light scattering biosensing. *Lab on a Chip* **20**, 1461-1471 (2020).
161. C. Li, R. J. Samulski, Engineering adeno-associated virus vectors for gene therapy. *Nat Rev Genet* **21**, 255-272 (2020).
162. K. Dzierlega, T. Yokota, Optimization of antisense-mediated exon skipping for Duchenne muscular dystrophy. *Gene Ther* **27**, 407-416 (2020).
163. L. N. Alfano, J. S. Charleston, A. M. Connolly, L. Cripe, C. Donoghue, R. Dracker, J. Dworzak, H. Eliopoulos, D. E. Frank, S. Lewis, K. Lucas, J. Lynch, A. J. Milici, A. Flynt, E. Naughton, L. R. Rodino-Klapac, Z. Sahenk, F. J. Schnell, G. D. Young, J. R. Mendell, L. P. Lowes, Long-term treatment with eteplirsen in nonambulatory patients with Duchenne muscular dystrophy. *Medicine (Baltimore)* **98**, e15858 (2019).
164. Y. Echigoya, K. R. Q. Lim, N. Trieu, B. Bao, B. Miskew Nichols, M. C. Vila, J. S. Novak, Y. Hara, J. Lee, A. Touznik, K. Mamchaoui, Y. Aoki, S. Takeda, K. Nagaraju, V. Mouly, R. Maruyama, W. Duddy, T. Yokota, Quantitative Antisense Screening and Optimization for Exon 51 Skipping in Duchenne Muscular Dystrophy. *Mol Ther* **25**, 2561-2572 (2017).
165. Y. Echigoya, A. Nakamura, T. Nagata, N. Urasawa, K. R. Q. Lim, N. Trieu, D. Panesar, M. Kuraoka, H. M. Moulton, T. Saito, Y. Aoki, P. Iversen, P. Sazani, R. Kole, R. Maruyama, T. Partridge, S. Takeda, T. Yokota, Effects of systemic multiexon skipping with peptide-conjugated morpholinos in the heart of a dog model of Duchenne muscular dystrophy. *Proc Natl Acad Sci U S A* **114**, 4213-4218 (2017).
166. A. Aiuti, M. G. Roncarolo, L. Naldini, Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. *EMBO Mol Med* **9**, 737-740 (2017).
167. C. H. Evans, S. C. Ghivizzani, P. D. Robbins, Gene Delivery to Joints by Intra-Articular Injection. *Hum Gene Ther* **29**, 2-14 (2018).
168. D. Bellavia, F. Veronesi, V. Carina, V. Costa, L. Raimondi, A. De Luca, R. Alessandro, M. Fini, G. Giavaresi, Gene therapy for chondral and osteochondral regeneration: is the future now? *Cell Mol Life Sci* **75**, 649-667 (2018).
169. X. Wang, Y. L. Li, R. Han, C. He, G. L. Wang, J. W. Wang, J. L. Zheng, M. Pei, L. Wei, Demineralized Bone Matrix Combined Bone Marrow Mesenchymal Stem Cells, Bone Morphogenetic Protein-2 and Transforming Growth Factor-beta 3 Gene Promoted Pig Cartilage Defect Repair (vol 9, e116061, 2014). *Plos One* **10**, (2015).
170. T. J. Sarkar, M. Quarta, S. Mukherjee, A. Colville, P. Paine, L. Doan, C. M. Tran, C. R. Chu, S. Horvath, L. S. Qi, N. Bhutani, T. A. Rando, V. Sebastiano, Transient non-integrative expression of nuclear reprogramming factors promotes multifaceted amelioration of aging in human cells. *Nat Commun* **11**, 1545 (2020).
171. L. Morales, Y. Gambhir, J. Bennett, H. H. Stedman, Broader Implications of Progressive Liver Dysfunction and Lethal Sepsis in Two Boys following Systemic High-Dose AAV. *Mol Ther* **28**, 1753-1755 (2020).
172. J. R. Mendell, K. Campbell, L. Rodino-Klapac, Z. Sahenk, C. Shilling, S. Lewis, D. Bowles, S. Gray, C. Li, G. Galloway, V. Malik, B. Coley, K. R. Clark, J. Li, X. Xiao, J. Samulski, S. W. McPhee, R. J. Samulski, C. M. Walker, Dystrophin immunity in Duchenne's muscular dystrophy. *N Engl J Med* **363**, 1429-1437 (2010).
173. J. Weinmann, S. Weis, J. Sippel, W. Tulalamba, A. Remes, J. El Andari, A. K. Herrmann, Q. H. Pham, C. Borowski, S. Hille, T. Schonberger, N. Frey, M. Lenter, T. VandenDriessche, O. J. Muller, M. K. Chuah, T. Lamla, D. Grimm, Identification of a myotropic AAV by massively parallel in vivo evaluation of barcoded capsid variants. *Nat Commun* **11**, 5432 (2020).
174. M. Tabebordbar, K. A. Lagerborg, A. Stanton, E. M. King, S. Ye, L. Tellez, A. Krunnfusz, S. Tavakoli, J. J. Widrick, K. A. Messemer, E. C. Troiano, B. Moghadaszadeh, B. L. Peacker, K. A. Leacock, N. Horwitz, A. H. Beggs, A. J. Wagers, P. C. Sabeti, Directed evolution of a family of AAV capsid variants enabling potent muscle-directed gene delivery across species. *Cell* **184**, 4919-4938 e4922 (2021).
175. Y. S. Yang, J. Xie, S. Chaugule, D. Wang, J. M. Kim, J. Kim, P. W. L. Tai, S. K. Seo, E. Gravallese, G. Gao, J. H. Shim, Bone-Targeting AAV-Mediated Gene Silencing in Osteoclasts for Osteoporosis Therapy. *Mol Ther Methods Clin Dev* **17**, 922-935 (2020).

176. Y. Yue, X. Pan, C. H. Hakim, K. Kodippili, K. Zhang, J. H. Shin, H. T. Yang, T. McDonald, D. Duan, Safe and bodywide muscle transduction in young adult Duchenne muscular dystrophy dogs with adeno-associated virus. *Hum Mol Genet* **24**, 5880-5890 (2015).
177. A. Ishii, H. Okada, H. Hayashita-Kinoh, J. H. Shin, A. Tamaoka, T. Okada, S. Takeda, rAAV8 and rAAV9-Mediated Long-Term Muscle Transduction with Tacrolimus (FK506) in Non-Human Primates. *Mol Ther Methods Clin Dev* **18**, 44-49 (2020).
178. P. P. Ho, L. J. Lahey, F. Mourkioti, P. E. Kraft, A. Filareto, M. Brandt, K. E. G. Magnusson, E. E. Finn, J. S. Chamberlain, W. H. Robinson, H. M. Blau, L. Steinman, Engineered DNA plasmid reduces immunity to dystrophin while improving muscle force in a model of gene therapy of Duchenne dystrophy. *Proc Natl Acad Sci U S A* **115**, E9182-E9191 (2018).
179. J. B. Kwon, A. R. Ettyreddy, A. Vankara, J. D. Bohning, G. Devlin, S. D. Hauschka, A. Asokan, C. A. Gersbach, In Vivo Gene Editing of Muscle Stem Cells with Adeno-Associated Viral Vectors in a Mouse Model of Duchenne Muscular Dystrophy. *Mol Ther Methods Clin Dev* **19**, 320-329 (2020).
180. L. R. Rodino-Klapac, C. L. Montgomery, W. G. Bremer, K. M. Shontz, V. Malik, N. Davis, S. Sprinkle, K. J. Campbell, Z. Sahenk, K. R. Clark, C. M. Walker, J. R. Mendell, L. G. Chicoine, Persistent expression of FLAG-tagged micro dystrophin in nonhuman primates following intramuscular and vascular delivery. *Mol Ther* **18**, 109-117 (2010).
181. J. R. Mendell, Z. Sahenk, K. Lehman, C. Nease, L. P. Lowes, N. F. Miller, M. A. Iammarino, L. N. Alfano, A. Nicholl, S. Al-Zaidy, S. Lewis, K. Church, R. Shell, L. H. Cripe, R. A. Potter, D. A. Griffin, E. Pozsgai, A. Dugar, M. Hogan, L. R. Rodino-Klapac, Assessment of Systemic Delivery of rAAVrh74.MHCK7.micro-dystrophin in Children With Duchenne Muscular Dystrophy: A Nonrandomized Controlled Trial. *JAMA Neurol* **77**, 1122-1131 (2020).
182. C. E. Winbanks, K. L. Weeks, R. E. Thomson, P. V. Sepulveda, C. Beyer, H. Qian, J. L. Chen, J. M. Allen, G. I. Lancaster, M. A. Febbraio, C. A. Harrison, J. R. McMullen, J. S. Chamberlain, P. Gregorevic, Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. *J Cell Biol* **197**, 997-1008 (2012).
183. S. A. Al-Zaidy, Z. Sahenk, L. R. Rodino-Klapac, B. Kaspar, J. R. Mendell, Follistatin Gene Therapy Improves Ambulation in Becker Muscular Dystrophy. *J Neuromuscul Dis* **2**, 185-192 (2015).
184. J. R. Mendell, Z. Sahenk, S. Al-Zaidy, L. R. Rodino-Klapac, L. P. Lowes, L. N. Alfano, K. Berry, N. Miller, M. Yalvac, I. Dvorchik, M. Moore-Clingenpeel, K. M. Flanigan, K. Church, K. Shontz, C. Curry, S. Lewis, M. McColly, M. J. Hogan, B. K. Kaspar, Follistatin Gene Therapy for Sporadic Inclusion Body Myositis Improves Functional Outcomes. *Mol Ther* **25**, 870-879 (2017).
185. Y. S. Yang, J. Xie, D. Wang, J. M. Kim, P. W. L. Tai, E. Gravallese, G. Gao, J. H. Shim, Bone-targeting AAV-mediated silencing of Schnurri-3 prevents bone loss in osteoporosis. *Nat Commun* **10**, 2958 (2019).
186. G. Wang, C. H. Evans, J. M. Benson, J. A. Hutt, J. Seagrave, J. A. Wilder, J. C. Grieger, R. J. Samulski, P. S. Terse, Safety and biodistribution assessment of sc-rAAV2.5IL-1Ra administered via intra-articular injection in a mono-iodoacetate-induced osteoarthritis rat model. *Mol Ther Methods Clin Dev* **3**, 15052 (2016).
187. M. Cucchiarini, H. Madry, Biomaterial-guided delivery of gene vectors for targeted articular cartilage repair. *Nat Rev Rheumatol* **15**, 18-29 (2019).
188. H. Frangoul, D. Altshuler, M. D. Cappellini, Y. S. Chen, J. Domm, B. K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T. W. Ho, A. Kattamis, A. Kernytsky, J. Lekstrom-Himes, A. M. Li, F. Locatelli, M. Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M. H. Steinberg, D. Wall, A. Yen, S. Corbacioglu, CRISPR-Cas9 Gene Editing for Sickle Cell Disease and beta-Thalassemia. *N Engl J Med* **384**, 252-260 (2021).
189. L. Amoasii, J. C. W. Hildyard, H. Li, E. Sanchez-Ortiz, A. Mireault, D. Caballero, R. Harron, T. R. Stathopoulou, C. Massey, J. M. Shelton, R. Bassel-Duby, R. J. Piercy, E. N. Olson, Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. *Science* **362**, 86-91 (2018).
190. C. E. Nelson, Y. Wu, M. P. Gemberling, M. L. Oliver, M. A. Waller, J. D. Bohning, J. N. Robinson-Hamm, K. Bulaklak, R. M. Castellanos Rivera, J. H. Collier, A. Asokan, C. A. Gersbach, Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy. *Nat Med* **25**, 427-432 (2019).
191. G. P. Freitas, H. B. Lopes, A. T. P. Souza, M. P. O. Gomes, G. K. Quiles, J. Gordon, C. Tye, J. L. Stein, G. S. Stein, J. E. B. Lian, M. M. Beloti, A. L. Rosa, Mesenchymal stem cells overexpressing BMP-9 by CRISPR-Cas9 present high in vitro osteogenic potential and enhance in vivo bone formation. *Gene Therapy*, (2021).

192. J. M. Brunger, A. Zutshi, V. P. Willard, C. A. Gersbach, F. Guilak, Genome Engineering of Stem Cells for Autonomously Regulated, Closed-Loop Delivery of Biologic Drugs. *Stem Cell Reports* **8**, 1202-1213 (2017).
193. Y. R. Choi, K. H. Collins, L. E. Springer, L. Pferdehirt, A. K. Ross, C. L. Wu, F. T. Moutos, N. S. Harasymowicz, J. M. Brunger, C. T. N. Pham, F. Guilak, A genome-engineered bioartificial implant for autoregulated anticytokine drug delivery. *Science Advances* **7**, (2021).
194. Y. L. Min, H. Li, C. Rodriguez-Caycedo, A. A. Mireault, J. Huang, J. M. Shelton, J. R. McAnally, L. Amoasii, P. P. A. Mammen, R. Bassel-Duby, E. N. Olson, CRISPR-Cas9 corrects Duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. *Sci Adv* **5**, eaav4324 (2019).
195. Y. Zhang, H. Li, Y. L. Min, E. Sanchez-Ortiz, J. Huang, A. A. Mireault, J. M. Shelton, J. Kim, P. P. A. Mammen, R. Bassel-Duby, E. N. Olson, Enhanced CRISPR-Cas9 correction of Duchenne muscular dystrophy in mice by a self-complementary AAV delivery system. *Sci Adv* **6**, eaay6812 (2020).
196. L. Amoasii, C. Long, H. Li, A. A. Mireault, J. M. Shelton, E. Sanchez-Ortiz, J. R. McAnally, S. Bhattacharyya, F. Schmidt, D. Grimm, S. D. Hauschka, R. Bassel-Duby, E. N. Olson, Single-cut genome editing restores dystrophin expression in a new mouse model of muscular dystrophy. *Sci Transl Med* **9**, (2017).
197. C. Long, H. Li, M. Tiburcy, C. Rodriguez-Caycedo, V. Kyrychenko, H. Zhou, Y. Zhang, Y. L. Min, J. M. Shelton, P. P. A. Mammen, N. Y. Liaw, W. H. Zimmermann, R. Bassel-Duby, J. W. Schneider, E. N. Olson, Correction of diverse muscular dystrophy mutations in human engineered heart muscle by single-site genome editing. *Sci Adv* **4**, eaap9004 (2018).
198. P. Marks, S. Gottlieb, Balancing Safety and Innovation for Cell-Based Regenerative Medicine. *N Engl J Med* **378**, 954-959 (2018).
199. B. T. O'Donnell, C. J. Ives, O. A. Mohiuddin, B. A. Bunnell, Beyond the Present Constraints That Prevent a Wide Spread of Tissue Engineering and Regenerative Medicine Approaches. *Front Bioeng Biotechnol* **7**, 95 (2019).
200. N. Naghshineh, S. Brown, P. S. Cederna, B. Levi, J. Lisiecki, R. A. D'Amico, K. M. Hume, W. Seward, J. P. Rubin, Demystifying the U.S. Food and Drug Administration: understanding regulatory pathways. *Plast Reconstr Surg* **134**, 559-569 (2014).
201. M. B. Leick, M. V. Maus, M. J. Frigault, Clinical Perspective: Treatment of Aggressive B Cell Lymphomas with FDA-Approved CAR-T Cell Therapies. *Mol Ther* **29**, 433-441 (2021).
202. D. Coyle, I. Durand-Zaleski, J. Farrington, L. Garrison, J. M. Graf von der Schulenburg, W. Greiner, L. Longworth, A. Meunier, A. S. Moutie, S. Palmer, Z. Pemberton-Whiteley, M. Ratcliffe, J. Shen, D. Sproule, K. Zhao, K. Shah, HTA methodology and value frameworks for evaluation and policy making for cell and gene therapies. *Eur J Health Econ* **21**, 1421-1437 (2020).
203. V. K. Rathi, J. S. Ross, Modernizing the FDA's 510(k) Pathway. *New Engl J Med* **381**, 1891-1893 (2019).
204. M. N. Doulgkeroglou, A. Di Nubila, B. Niessing, N. Konig, R. H. Schmitt, J. Damen, S. J. Szilvassy, W. Chang, L. Csontos, S. Louis, P. Kugelmeier, V. Ronfard, Y. Bayon, D. I. Zeugolis, Automation, Monitoring, and Standardization of Cell Product Manufacturing. *Front Bioeng Biotechnol* **8**, 811 (2020).
205. T. Wei, Q. Cheng, Y. L. Min, E. N. Olson, D. J. Siegwart, Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nat Commun* **11**, 3232 (2020).
206. C. D. Sago, M. P. Lokugamage, F. Z. Islam, B. R. Krupczak, M. Sato, J. E. Dahlman, Nanoparticles That Deliver RNA to Bone Marrow Identified by in Vivo Directed Evolution. *J Am Chem Soc* **140**, 17095-17105 (2018).
207. K. Elverum, M. Whitman, Delivering cellular and gene therapies to patients: solutions for realizing the potential of the next generation of medicine. *Gene Ther* **27**, 537-544 (2020).
208. M. Hay, D. W. Thomas, J. L. Craighead, C. Economides, J. Rosenthal, Clinical development success rates for investigational drugs. *Nat Biotechnol* **32**, 40-51 (2014).
209. A. Skardal, S. V. Murphy, M. Devarasetty, I. Mead, H. W. Kang, Y. J. Seol, Y. Shrike Zhang, S. R. Shin, L. Zhao, J. Aleman, A. R. Hall, T. D. Shupe, A. Kleensang, M. R. Dokmeci, S. Jin Lee, J. D. Jackson, J. J. Yoo, T. Hartung, A. Khademhosseini, S. Soker, C. E. Bishop, A. Atala, Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform. *Sci Rep* **7**, 8837 (2017).
210. M. J. C. de Kok, A. F. Schaapherder, R. C. I. Wust, M. Zuiderwijk, J. A. Bakker, J. H. N. Lindeman, S. E. Le Devedec, Circumventing the Crabtree effect in cell culture: A systematic review. *Mitochondrion* **59**, 83-95 (2021).

211. J. R. Cantor, M. Abu-Remaileh, N. Kanarek, E. Freinkman, X. Gao, A. Louissaint, Jr., C. A. Lewis, D. M. Sabatini, Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase. *Cell* **169**, 258-272 e217 (2017).
212. A. Polini, L. L. Del Mercato, A. Barra, Y. S. Zhang, F. Calabi, G. Gigli, Towards the development of human immune-system-on-a-chip platforms. *Drug Discov Today* **24**, 517-525 (2019).
213. E. Shorter, A. J. Sannicandro, B. Poulet, K. Goljanek-Whysall, Skeletal Muscle Wasting and Its Relationship With Osteoarthritis: a Mini-Review of Mechanisms and Current Interventions. *Curr Rheumatol Rep* **21**, 40 (2019).
214. T. M. Allen, M. A. Brehm, S. Bridges, S. Ferguson, P. Kumar, O. Mirochnitchenko, K. Palucka, R. Pelanda, B. Sanders-Beer, L. D. Shultz, L. Su, M. PrabhuDas, Humanized immune system mouse models: progress, challenges and opportunities. *Nat Immunol* **20**, 770-774 (2019).
215. I. Ribitsch, P. M. Baptista, A. Lange-Consiglio, L. Melotti, M. Patruno, F. Jenner, E. Schnabl-Feichter, L. C. Dutton, D. J. Connolly, F. G. van Steenbeek, J. Dudhia, L. C. Penning, Large Animal Models in Regenerative Medicine and Tissue Engineering: To Do or Not to Do. *Front Bioeng Biotechnol* **8**, 972 (2020).