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tissue engineering of functional skeletal muscle: challenges and recent advances

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Self-repair capacity of the adult skeletal muscle is deficient in its ability to restore significant tissue loss caused by traumatic injury, congenital defects, tumor ablation, prolonged denervation, or functional damage due to a variety of myopathies [1], [2]. Conventional surgical treatments including local or distant autologous muscle transposition yield a limited degree of success [2]. Alternatively, transplantation of exogenous myogenic cells (satellite cells and myoblasts) has been proposed to increase the regenerative capacity of skeletal muscle [3]. However, the clinical outcomes from intramuscular injection of allogeneic myoblasts were compromised by numerous limitations, including poor cell retention and survival, as well as immunorejection [3], [4]. Studies of other muscle-derived stem cells [5] and genetically modified myoblasts [6] are currently in progress, for their ability to overcome these limitations and improve therapeutic efficacy.

On the other hand, the emergence of tissue-engineering technology in the past decade presents another attractive treatment option, where engineered tissue substitutes could be used for the functional and aesthetic reconstruction of damaged skeletal muscle [2]. Compared with the delivery of isolated muscle precursor cells in a suspension or a temporary matrix, the in vitro fabrication of mature artificial muscle may provide unique therapeutic benefits including 1) the ability to preengineer custom tissue architecture for precise structural repair at the site of injury (e.g., for craniofacial reconstruction), 2) the ability to precondition tissue implants for specific mechanically or metabolically demanding host environments such as sarcopenic muscle in the elderly or site of traumatic injury, and 3) localized delivery of concentrated angiogenic and antiapoptotic paracrine factors upon implantation. However, current tissue-engineered muscle constructs lack the morphology, ultrastructure, and level of differentiation found in native adult muscle. Typically, these constructs consist of short length, small diameter, premature myofibers packed at low density within a small volume and, thus, generate forces more than an order of magnitude lower than normal adult muscle, thereby providing little or no clinical utility [7]. This article briefly reviews a few important technical challenges in the field and describes current and potential strategies to advance the engineering of functional skeletal muscle toward potential clinical use in the repair of muscle damage.

Challenge 1: Fabrication of Dense Skeletal Muscle Tissues with Adequate Dimensions, Uniform Cell Alignment, and Reproducible Architecture

From a biomimetic perspective, functional engineered skeletal muscle tissues should exhibit native-like structural properties and, specifically, contain densely packed and uniformly aligned myofibers throughout a relatively large tissue volume. High muscle cell density and alignment was previously achieved by constraining the cell growth within thin and long muscle bundles using centrifugal cell packing in cylindrically shaped collagen gels [8] and self-organization of cells in scaffold-free myooids under passive tension [9]. Recently, engineering of dense, aligned, 100–150-μm thick artificial muscle tissues with a relatively large area (a few square centimeters) was attempted by successively layering collagen matrix and seeding myoblasts in a Petri dish [10]. It was not certain, however, whether the myoblasts within the top layers of this multilayer structure coaligned with myoblasts oriented along the direction of

brushed collagen at the bottom of the dish. Aligned porous structures have also been fabricated using relatively thick (2–2.5 mm) polymeric scaffolds made of collagen [11] or poly(lactic-*co*-glycolic) acid [12] in an attempt to orient muscle cells in a three-dimensional (3-D) manner. Nevertheless, reliable methods to uniformly and densely align muscle cells within a relatively large and thick 3-D tissue construct are still lacking. In particular, the inability to provide sufficient oxygen and nutrient supply to match the high metabolic demand of active muscle tissue can result in the formation of a necrotic core inside the thick constructs [13]. Moreover, current muscle tissue-engineering methods lack the structural and functional reproducibility that is essential for a future off-the-shelf supply of standardized engineered tissues.

One of the promising approaches for the biomimetic design of engineered muscle is the use of biocompatible hydrogels. Although natural hydrogels (e.g., collagen [8], [14], matrigel [14], and fibrin [9], [15]) usually exhibit higher batch-to-batch variability and are less amenable to the control of their physicochemical properties than synthetic hydrogels, they still appear superior for muscle tissue engineering primarily due to a higher density of cell attachment sites necessary for 3-D cell spreading. In addition, hydrogels also appear superior to polymeric materials for the engineering of highly cellular soft tissues, such as muscle, because they allow for spatially uniform and dense cell entrapment [16], higher ultimate cell density due to significant cell-mediated gel compaction [17], [18], and control of cell alignment through the application of mechanical forces [19], [20]. One of the main disadvantages of hydrogel use has been the difficulty in fabricating tissue constructs with arbitrary 3-D shapes. However, photo-lithographic [16], [21] and soft-lithographic [22], [23] patterning of hydrogels have been applied recently for the engineering of complex hepatic and vascular tissue structures [24], [25]. These rapid prototyping techniques [26] enable reproducible control of hydrogel geometry and, by initially confining one or several cell types in the desired 3-D configuration, may facilitate the engineering of customized tissue architectures. In addition, they may allow for systematic control of the porosity and pore inter-connectivity within constructs [27], [28] and the layered assembly of 3-D objects [16], [28], both of which could increase the attainable thickness of viable and dense engineered muscle. The capability of computer-aided design may further permit the fabrication of complex muscle tissue structures with high accuracy and repeatability [28].

Challenge 2: In Vitro Maximization of Active Force Generation by Improved Cell Growth, Enhanced Differentiation, and Optimized 3-D Cell-Matrix Interactions

Engineered skeletal muscle tissues are expected to generate sufficiently large active forces to be able to restore the impaired host muscle function upon transplantation. The force production capability of engineered muscle can be enhanced not only by creating the native-like tissue architecture but also by promoting the growth and differentiation of cells into fully mature muscle fibers and optimizing the interactions that these fibers have with the surrounding 3-D matrix.

Mechanical stretch has long been known to induce a significant increase in protein synthesis and stimulate skeletal muscle growth in vitro and in vivo [29]. In particular, sustained passive tension has been shown to regulate 3-D cell spreading inside a collagen matrix [30] and, when applied uniaxially, facilitate the alignment and fusion of myoblasts into multinucleated myotubes [30], [31]. Cyclic strain has been shown to activate quiescent satellite cell populations [32], [33] and increase the proliferation rate of myoblasts [34], [35]. Applying specific patterns of mechanical stimulation also improved the force production of tissue-engineered skeletal muscle by increasing myofiber diameter and density [14], [36]. In addition, the repetitive stretch of muscle tissue constructs inhibited collagen cross-linking and stiffening

with time in culture yielding higher-tissue elasticity than that obtained in static cultures [14]. On the other hand, inconsistent findings have been reported regarding the role of mechanical stretch (promotion versus inhibition) on various aspects of muscle differentiation including the expression of myogenic regulatory factors (e.g., MyoD and MNF-β) [34], [37] and muscle-specific proteins [e.g., myosin heavy chain (MHC)] [38], [39] and the formation of multinucleated myotubes [34], [39], [40]. Although the underlying molecular mechanisms of mechanotransduction remain to be elucidated, the specific parameters of cyclic strain such as amplitude, frequency, and rest period may play important roles in the fine control of the balance between cell proliferation and differentiation. Besides mechanical stretch, the application of electrical stimulation to two-dimensional (2-D) myoblast cultures has been shown to facilitate sarcomere assembly and myofiber maturation through induced contractile activity [41], [42]. In particular, electrically induced intracellular Ca^{2+} oscillations at selected stimulation frequencies accelerated the development of mature sarcomeric structures (e.g., Z lines and A bands) in differentiating myotubes [41]. The specific electrical stimulus patterns (with different intraburst and interburst frequencies) caused specific changes in the expression of fast and slow MHC isoforms [43]. Whether electrical stimulation can promote the differentiation and maturation of 3-D engineered skeletal muscle remains to be investigated. It is possible that combined electrical and mechanical stimulation regimes will allow for an independent control of cell proliferation and differentiation during tissue-engineered myogenesis.

The growth and differentiation of muscle cells and, consequently, force production could also be improved by finely tuning the 3-D biochemical and mechanical microenvironment within engineered tissues. Specific myogenic molecules including different growth factors, cytokines, and genes (or combinations of these molecules) could be incorporated into hydrogels or polymer scaffolds [44], [45] and released in the cell vicinity in a temporally controlled and spatially uniform fashion [26], [46]. For example, the insulin-like growth factors (IGFs) 1 and 2 are known to regulate myogenesis by controlling proliferation and differentiation of myoblasts via different signaling pathways [47]. The addition of IGF-1 to cell culture media has been shown recently to promote the differentiation, hypertrophy, and force generation of tissue-engineered muscle [48], [49]. In addition, the mechanical compliance of the surrounding matrix may play a significant role in striated muscle differentiation. It has been demonstrated that optimal myotube differentiation occurred on 2-D substrates with a stiffness similar to that of native muscle [50]. Although a clear understanding of how to translate the concept of 2-D substrate stiffness into a 3-D tissue environment is still lacking, the ability to tailor engineered-tissue stiffness through changes in hydrogel chemistry [51] may offer additional routes toward enhancing the differentiation of engineered muscle.

It is important to note that the smaller myotube size and density within engineered tissues, relative to those found in native muscle, are expected to amplify the role that cell-matrix interactions play in the generation and transmission of force [52]. The tissue-engineered muscle is likely to behave as native series-fibered muscles where both in-series and in-parallel connections of short myofibers to the surrounding matrix and to other fibers significantly contribute to the total force generation [53], [54]. Therefore, for the same cell density and alignment, the passive (viscoelastic) and active (contractile) mechanical properties of the muscle constructs will strongly depend on the number and strength of cell-matrix adhesions. Thus, to enhance the force-generating capability of engineered muscle, it will be important to understand the biochemical and physical factors that regulate the expression and assembly of costamere-associated proteins (e.g., dystrophin, α- and β-dystroglycan, and syntrophin) that link the skeletal muscle to the extracellular matrix and transmit forces transversely [55] as well as different focal adhesion proteins localized in the myotendinous junctions (e.g., $\alpha 7$ integrins, paxillin, vinculin, and talin) that are known to participate in longitudinal force transmission [52], [56].

Challenge 3: Rapid Vascularization and Innervation to Promote Long-Term Survival and functional Donor-Host Integration upon In Vivo Implantation

Engineering a patent vascular bed inside a tissue construct remains the holy grail of tissue engineering because it would permit the improved oxygen and nutrient exchange needed to generate thick tissues in vitro and the immediate connection of the tissue graft to the recipient circulation to prevent hypoxia-induced cell damage after transplantation. Although no current tissue-engineering methods can achieve this goal, intensive research efforts in recent years have led to a number of promising approaches that could be used alone or in combination to promote vascularization of skeletal muscle constructs. For example, coculture of C2C12 myoblasts, embryonic fibroblasts, and endothelial cells on highly porous and biodegradable polymer scaffolds resulted in the formation of endothelial networks within engineered muscle tissues in vitro and enhanced vascularization, blood perfusion, and survival of the tissue constructs after implantation [57]. Other methods for vascularization include the formation of engineered muscle tissue within or around the in vivo perfusion systems such as the arterio-venous loop or femoral artery [58], [59]. Furthermore, the addition of angiogenic factors either by genetic alteration of donor cells [60], [61] or by controlled release from bio-active scaffolds [62] may additionally facilitate in vivo vascularization of the engineered tissue grafts. For instance, the use of genetically modified myoblasts expressing vascular endothelial growth factor (VEGF) has been shown to increase neovascularization and tissue mass of in vivo engineered muscle [60].

Finally, the ability of engineered muscle tissues to rapidly connect to the host neuromuscular system is expected to further facilitate their functional integration into the host environment and accelerate the functional recovery of the host muscle. A number of previous in vivo studies have demonstrated muscle atrophy after long-term denervation and the ability of chronic electrical stimulation to partially reverse this effect [63]–[65]. Recent in vitro investigations on nerve-skeletal muscle constructs have shed additional light on the role of innervation in muscle differentiation and function and the potential of engineered muscles to be innervated in culture. In particular, coculturing muscle constructs with neural cells [15], [66] or inducing construct neurotization using transected nerves [67] not only enhanced the differentiation and force generation of muscle cells but also yielded the formation of acetylcholine-sensitive neuromuscular junctions and allowed indirect muscle stimulation via the neural extensions projecting from the tissue constructs. Further implantation studies with electrically stimulated or cocultured nerve-muscle constructs are expected to reveal the potential benefits of these approaches to in vivo innervation of engineered muscle tissues and their functional integration into the host muscle.

Conclusions

The use of engineered tissue substitutes to restore lost skeletal muscle function in a variety of pathological conditions is hampered by the lack of methods to 1) fabricate muscle constructs with native tissue architecture and sufficient force generation capability and 2) prevent poor survival and integration of tissue constructs into the host muscle. Recent advancements in the fields of tissue engineering and muscle stem cell biology are expected to facilitate the development of innovative strategies that could overcome these limitations. Patterned hydrogels produced by novel rapid prototyping techniques could be used as scaffolds for large and thick engineered muscle tissues made of uniformly aligned and densely packed myofibers. The active force generated by the engineered muscle could be maximized by promoting muscle differentiation and maturation via the application of appropriate regimes of electrical and mechanical stimulation and/or the use of growth factors. The design of favorable biochemical and mechanical microenvironments to enhance cell-matrix interactions may further improve cell attachment, survival, differentiation, and force generation. Various approaches have also

been suggested to facilitate rapid vascularization and innervation of tissue constructs upon transplantation, which could benefit their long-term survival and functional integration into the host tissue. The recent progress in the field of skeletal muscle tissue engineering along with the continued effort to evaluate novel cell transplantation sources holds potential for the development of an effective clinical therapy for the restoration of compromised muscle function.

References

1. Alsberg E, Hill EE, Mooney DJ. Craniofacial tissue engineering. Crit Rev Oral Biol Med 2001;12:64–75. [PubMed: 11349963]
2. Bach AD, Beier JP, Stern-Staeter J, Horch RE. Skeletal muscle tissue engineering. J Cell Mol Med Oct./Dec.;2004 8:413–422. [PubMed: 15601570]
3. Vilquin JT. Myoblast transplantation: Clinical trials and perspectives. A mini review. Acta Myol Oct.; 2005 24:119–127. [PubMed: 16550929]
4. Mouly V, Aamiri A, Perie S, Mamchaoui K, Barani A, Bigot A, Bouazza B, Francois V, Furling D, Jacquemin V, Negroni E, Riederer I, Vignaud A, Guily JL, Butler-Browne GS. Myoblast transfer therapy: Is there any light at the end of the tunnel? Acta Myol Oct.;2005 24:128–133. [PubMed: 16550930]
5. Urish K, Kanda Y, Huard J. Initial failure in myoblast transplantation therapy has led the way toward the isolation of muscle stem cells: Potential for tissue regeneration. Curr Top Dev Biol 2005;68:263–280. [PubMed: 16125002]
6. Qu Z, Balkir L, van Deutekom JC, Robbins PD, Pruchnic R, Huard J. Development of approaches to improve cell survival in myoblast transfer therapy. J Cell Biol Sept.;1998 142:1257–1267. [PubMed: 9732286]
7. Vandenburgh HH. Functional assessment and tissue design of skeletal muscle. Ann NY Acad Sci 2002;961:201–202. [PubMed: 12081899]
8. Okano T, Matsuda T. Tissue engineered skeletal muscle: Preparation of highly dense, highly oriented hybrid muscular tissues. Cell Transplant Jan./Feb.;1998 7:71–82. [PubMed: 9489765]
9. Huang YC, Dennis RG, Larkin L, Baar K. Rapid formation of functional muscle in vitro using fibrin gels. J Appl Physiol Feb.;2005 98:706–713. [PubMed: 15475606]
10. Yan W, George S, Fotadar U, Tyhovych N, Kamer A, Yost MJ, Price RL, Haggart CR, Holmes JW, Terracio L. Tissue engineering of skeletal muscle. Tissue Eng. Sept.;2007
11. Kroehne V, Heschel I, Schugner F, Lasrich D, Bartsch JW, Jockusch H. Use of a novel collagen matrix with oriented pore structure for muscle cell differentiation in cell culture and in grafts. J Cell Mol Med 2008;12:1582–1838.
12. Bursac N, Loo Y, Leong K, Tung L. Novel anisotropic engineered cardiac tissues: studies of electrical propagation. Biochem Biophys Res Commun Oct.;2007 361:847–853. [PubMed: 17689494]
13. Davis BH, Schroeder T, Yarmolenko PS, Guilak F, Dewhirst MW, Taylor DA. An in vitro system to evaluate the effects of ischemia on survival of cells used for cell therapy. Ann Biomed Eng Aug.; 2007 35:1414–1424. [PubMed: 17417737]
14. Powell CA, Smiley BL, Mills J, Vandenburgh HH. Mechanical stimulation improves tissue-engineered human skeletal muscle. Am J Physiol Cell Physiol Nov.;2002 283:C1557–C1565. [PubMed: 12372817]
15. Bach AD, Beier JP, Stark GB. Expression of Trisk 51, agrin and nicotinic-acety-choline receptor epsilon-subunit during muscle development in a novel three-dimensional muscle-neuronal co-culture system. Cell Tissue Res Nov.;2003 314:263–274. [PubMed: 12955494]
16. Tsang VL, Bhatia SN. Three-dimensional tissue fabrication. Adv Drug Deliv Rev Sept.;2004 56:1635–1647. [PubMed: 15350293]
17. Rowe SL, Stegemann JP. Interpenetrating collagen-fibrin composite matrices with varying protein contents and ratios. Biomacromolecules Nov.;2006 7:2942–2948. [PubMed: 17096517]
18. Shi Y, Rittman L, Vesely I. Novel geometries for tissue-engineered tendonous collagen constructs. Tissue Eng. 2006

19. Costa KD, Lee EJ, Holmes JW. Creating alignment and anisotropy in engineered heart tissue: Role of boundary conditions in a model three-dimensional culture system. Tissue Eng Aug.;2003 9:567–577. [PubMed: 13678436]
20. Girton TS, Barocas VH, Tranquillo RT. Confined compression of a tissue-equivalent: Collagen fibril and cell alignment in response to anisotropic strain. J Biomech Eng Oct.;2002 124:568–575. [PubMed: 12405600]
21. Hahn MS, Taite LJ, Moon JJ, Rowland MC, Ruffino KA, West JL. Photolithographic patterning of polyethylene glycol hydrogels. Biomaterials Apr.;2006 27:2519–2524. [PubMed: 16375965]
22. Tang MD, Golden AP, Tien J. Molding of three-dimensional microstructures of gels. J Am Chem Soc Oct.;2003 125:12988–12989. [PubMed: 14570447]
23. Norman JJ, Desai TA. Control of cellular organization in three dimensions using a micro-fabricated polydimethylsiloxane-collagen composite tissue scaffold. Tissue Eng Mar./Apr.;2005 11:378–386. [PubMed: 15871668]
24. Liu Tsang V, Chen AA, Cho LM, Jadin KD, Sah RL, DeLong S, West JL, Bhatia SN. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. Faseb J Mar.;2007 21:790–801. [PubMed: 17197384]
25. Chrobak KM, Potter DR, Tien J. Formation of perfused, functional microvascular tubes in vitro. Microvasc Res May;2006 71:185–196. [PubMed: 16600313]
26. Fedorovich NE, Alblas J, de Wijn JR, Hennink WE, Verbout AJ, Dhert WJ. Hydrogels as extracellular matrices for skeletal tissue engineering: State-of-the-art and novel application in organ printing. Tissue Eng Aug.;2007 13:1905–1925. [PubMed: 17518748]
27. Bryant SJ, Cuy JL, Hauch KD, Ratner BD. Photo-patterning of porous hydrogels for tissue engineering. Biomaterials Jul.;2007 28:2978–2986. [PubMed: 17397918]
28. Leong KF, Cheah CM, Chua CK. Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. Biomaterials June;2003 24:2363–2378. [PubMed: 12699674]
29. Taylor NA, Wilkinson JG. Exercise-induced skeletal muscle growth. Hypertrophy or hyperplasia? Sports Med May/June;1986 3:190–200. [PubMed: 3520748]
30. Vandenburgh HH, Karlisch P. Longitudinal growth of skeletal myotubes in vitro in a new horizontal mechanical cell stimulator. In Vitro Cell Dev Biol 1989;25:607–616. [PubMed: 2753848]
31. Cheema U, Yang SY, Mudera V, Gold-spink GG, Brown RA. 3-D in vitro model of early skeletal muscle development. Cell Motil Cytoskeleton Mar.;2003 54:226–236. [PubMed: 12589681]
32. Tatsumi R, Sheehan SM, Iwasaki H, Hattori A, Allen RE. Mechanical stretch induces activation of skeletal muscle satellite cells in vitro. Exp Cell Res 2001;267:107–114. [PubMed: 11412043]
33. Darr KC, Schultz E. Exercise-induced satellite cell activation in growing and mature skeletal muscle. J Appl Physiol Nov.;1987 63:1816–1821. [PubMed: 3693217]
34. Kook SH, Lee HJ, Chung WT, Hwang IH, Lee SA, Kim BS, Lee JC. Cyclic mechanical stretch stimulates the proliferation of C2C12 myoblasts and inhibits their differentiation via prolonged activation of p38 MAPK. Mol Cells Apr.;2008 25
35. Otis JS, Burkholder TJ, Pavlath GK. Stretch-induced myoblast proliferation is dependent on the COX2 pathway. Exp Cell Res 2005;310:417–425. [PubMed: 16168411]
36. Vandenburgh HH, Hatfaludy S, Karlisch P, Shansky J. Skeletal muscle growth is stimulated by intermittent stretch-relaxation in tissue culture. Am J Physiol Mar.;1989 256:C674–C682. [PubMed: 2923199]
37. Akimoto T, Ushida T, Miyaki S, Tateishi T, Fukubayashi T. Mechanical stretch is a down-regulatory signal for differentiation of C2C12 myogenic cells. Mater Sci Eng C 2001;17:75–78.
38. Rauch C, Loughna PT. Static stretch promotes MEF2A nuclear translocation and expression of neonatal myosin heavy chain in C2C12 myocytes in a calcineurin- and p38-dependent manner. Am J Physiol Cell Physiol Mar.;2005 288:C593–C605. [PubMed: 15483225]
39. Kumar A, Murphy R, Robinson P, Wei L, Boriek AM. Cyclic mechanical strain inhibits skeletal myogenesis through activation of focal adhesion kinase, Rac-1 GTPase, and NF-kappaB transcription factor. Faseb J Oct.;2004 18:1524–1535. [PubMed: 15466361]

40. Zhang SJ, Truskey GA, Kraus WE. Effect of cyclic stretch on beta1D-integrin expression and activation of FAK and RhoA. Am J Physiol Cell Physiol June;2007 292:C2057–C2069. [PubMed: 17267546]
41. Fujita H, Nedachi T, Kanzaki M. Accelerated de novo sarcomere assembly by electric pulse stimulation in C2C12 myotubes. Exp Cell Res May;2007 313:1853–1865. [PubMed: 17425954]
42. De Deyne PG. Formation of sarcomeres in developing myotubes: Role of mechanical stretch and contractile activation. Am J Physiol Cell Physiol Dec.;2000 279:C1801–C1811. [PubMed: 11078695]
43. Naumann K, Pette D. Effects of chronic stimulation with different impulse patterns on the expression of myosin isoforms in rat myotube cultures. Differentiation Feb.;1994 55:203–211. [PubMed: 8187982]
44. Lutolf MP, Hubbell JA. Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering. Nat Biotechnol Jan.;2005 23:47–55. [PubMed: 15637621]
45. Storrie H, Mooney DJ. Sustained delivery of plasmid DNA from polymeric scaffolds for tissue engineering. Adv Drug Deliv Rev July;2006 58:500–514. [PubMed: 16759734]
46. Cao L, Mooney DJ. Spatiotemporal control over growth factor signaling for therapeutic neovascularization. Adv Drug Deliv Rev Nov.;2007 59:1340–1350. [PubMed: 17868951]
47. Coolican SA, Samuel DS, Ewton DZ, McWade FJ, Florini JR. The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. J Biol Chem Mar.;1997 272:6653–6662. [PubMed: 9045696]
48. Gawlitta D, Boonen KJ, Oomens CW, Baaijens FP, Bouten CV. The influence of serum-free culture conditions on skeletal muscle differentiation in a tissue-engineered model. Tissue Eng Part A Jan.; 2008 14:161–171. [PubMed: 18333814]
49. Vandenburgh H, Shansky J, Benesch-Lee F, Barbata V, Reid J, Thorrez L, Valentini R, Crawford G. Drug-screening platform based on the contractility of tissue-engineered muscle. Muscle Nerve Apr.; 2008 37:438–447. [PubMed: 18236465]
50. Engler AJ, Griffin MA, Sen S, Bonnemann CG, Sweeney HL, Discher DE. Myotubes differentiate optimally on substrates with tissue-like stiffness: Pathological implications for soft or stiff microenvironments. J Cell Biol Sept.;2004 166:877–887. [PubMed: 15364962]
51. Brandl F, Sommer F, Goepferich A. Rational design of hydrogels for tissue engineering: Impact of physical factors on cell behavior. Biomaterials Jan.;2007 28:134–146. [PubMed: 17011028]
52. Monti RJ, Roy RR, Hodgson JA, Edgerton VR. Transmission of forces within mammalian skeletal muscles. J Biomech Apr.;1999 32:371–380. [PubMed: 10213027]
53. Young M, Paul A, Rodda J, Duxson M, Sheard P. Examination of intrafascicular muscle fiber terminations: Implications for tension delivery in series-fibered muscles. J Morphol Aug.;2000 245:130–145. [PubMed: 10906747]
54. Paul AC, Sheard PW, Kaufman SJ, Duxson MJ. Localization of alpha7 integrins and dystrophin suggests potential for both lateral and longitudinal transmission of tension in large mammalian muscles. Cell Tissue Res May;2002 308:255–265. [PubMed: 12037582]
55. Ervasti JM. Costameres: the Achilles' heel of Herculean muscle. J Biol Chem Apr.;2003 278:13591–13594. [PubMed: 12556452]
56. Tidball JG. Mechanical signal transduction in skeletal muscle growth and adaptation. J Appl Physiol May;2005 98:1900–1908. [PubMed: 15829723]
57. Levenberg S, Rouwkema J, Macdonald M, Garfein ES, Kohane DS, Darland DC, Marini R, van Blitterswijk CA, Mulligan RC, D'Amore PA, Langer R. Engineering vascularized skeletal muscle tissue. Nat Biotechnol July;2005 23:879–884. [PubMed: 15965465]
58. Messina A, Bortolotto SK, Cassell OC, Kelly J, Abberton KM, Morrison WA. Generation of a vascularized organoid using skeletal muscle as the inductive source. Faseb J Sept.;2005 19:1570–1572. [PubMed: 16014398]
59. Borschel GH, Dow DE, Dennis RG, Brown DL. Tissue-engineered axially vascularized contractile skeletal muscle. Plast Reconstr Surg June;2006 117:2235–2242. [PubMed: 16772923]
60. De Coppi P, Delo D, Farrugia L, Udompanyanan K, Yoo JJ, Nomi M, Atala A, Soker S. Angiogenic gene-modified muscle cells for enhancement of tissue formation. Tissue Eng July/Aug.;2005 11:1034–1044. [PubMed: 16144439]

61. Suzuki K, Murtuza B, Smolenski RT, Sammut IA, Suzuki N, Kaneda Y, Yacoub MH. Cell transplantation for the treatment of acute myocardial infarction using vascular endothelial growth factor-expressing skeletal myoblasts. Circulation Sept.;2001 104:1207–1212.

62. Zisch AH, Lutolf MP, Hubbell JA. Biopolymeric delivery matrices for angiogenic growth factors. Cardiovasc Pathol Nov./Dec.;2003 12:295–310. [PubMed: 14630296]

63. Boncompagni S, Kern H, Rossini K, Hofer C, Mayr W, Carraro U, Protasi F. Structural differentiation of skeletal muscle fibers in the absence of innervation in humans. Proc Natl Acad Sci USA Dec.; 2007 104:19339–19344. [PubMed: 18042706]

64. Ashley Z, Salmons S, Boncompagni S, Protasi F, Russold M, Lanmuller H, Mayr W, Sutherland H, Jarvis JC. Effects of chronic electrical stimulation on long-term denervated muscles of the rabbit hind limb. J Muscle Res Cell Motil 2007;28:203–217. [PubMed: 17906933]

65. Eberstein A, Eberstein S. Electrical stimulation of denervated muscle: Is it worthwhile? Med Sci Sports Exerc Dec.;1996 28:1463–1469. [PubMed: 8970139]

66. Larkin LM, Van der Meulen JH, Dennis RG, Kennedy JB. Functional evaluation of nerve-skeletal muscle constructs engineered in vitro. In Vitro Cell Dev Biol Anim Mar./Apr.;2006 42:75–82. [PubMed: 16759152]

67. Dhawan V, Lytle IF, Dow DE, Huang YC, Brown DL. Neurotization improves contractile forces of tissue-engineered skeletal muscle. Tissue Eng Nov.;2007 13:2813–2821. [PubMed: 17822360]