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Advances in myogenic cell transplantation and skeletal muscle tissue engineering

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1. ABSTRACT

Curative treatments are currently not available for people suffering from one of the many prevalent muscle myopathies. One approach to ameliorate these conditions relies on the cell-based transplantation of potential myogenic precursors, or more optimistically, the transfer of engineered skeletal muscle tissue. To date, clinical trials with myogenic stem cell transplantation have met with only modest success while the transplantation of engineered muscle tissue is at the earliest stages of development. Despite the slow progress, these studies have provided insights and avenues that will eventually lead to a powerful therapeutic tool.

2. INTRODUCTION

Adult skeletal muscle regeneration is a highly orchestrated process that involves the activation of muscle progenitor cells. Severe muscular trauma and genetic disorders such as muscular dystrophy cause a major draw-down and, ultimately, the exhaustion of these cells. On the other hand, the inability of myogenic cells to become activated and differentiate properly leads to extensive muscle wasting, as is the case in disease states characterized by cachexia as well as sarcopenia in the elderly. It is thus of utmost importance to find sources for potential myogenic precursors and develop methods for their use as therapeutic tools.

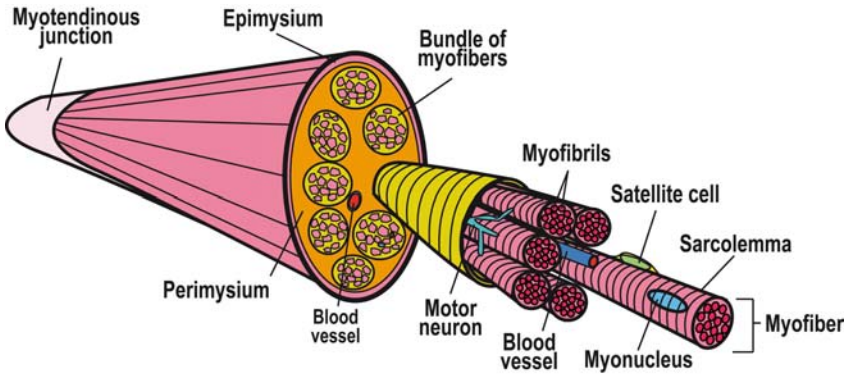

Figure 1. Structure of skeletal muscle. Skeletal muscle is formed by grouping bundles of myofibers within the perimysium, which is itself contained by the epimysium. Myofibers cross the entire muscle lengthwise and attach to the bone through myotendinous junctions. Myofibers are multinucleated single cells made up of an array of stacked myofibrils. The myofibrils consist of thick and thin filaments organized into a contractile unit called a sarcomere. The myofibers are surrounded by a basal lamina under which a population of quiescent muscle progenitor cells (satellite cells) are located. A rich network of blood vessels and nerves weaves between and within the myofibers.

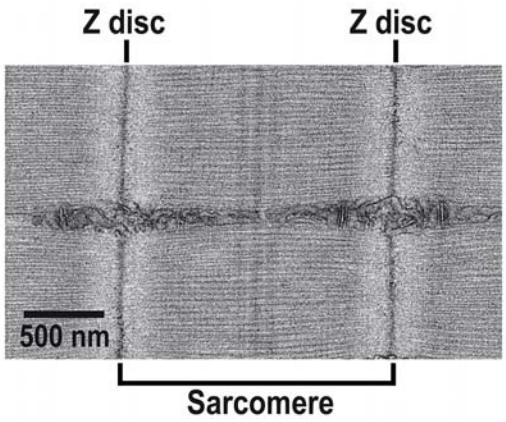

Figure 2. Transmission electron micrograph of a sarcomere. Transmission electron microscopic views of a skeletal muscle sarcomeric unit showing the uniform distribution of thin and thick microfilaments.

In recent years, the field of regenerative medicine has expanded exponentially following the discovery of stem cells, which possess an exceptional capacity to differentiate into a variety of tissues (1). The advantages of using stem cells with myogenic potential in treating myopathies are significant. The delivery of cells containing a normal genome can restock the pool of dysfunctional myogenic cells and reduce muscle wasting. In addition, because of their myogenic potential, stem cells are good candidates for engineering of skeletal muscle tissue *in vitro* for subsequent use *in vivo*.

In this chapter, we will briefly review the structure of the skeletal muscle and the molecular and cellular regulation mechanisms that lead to the formation and repair of muscle fibers. We will assess the literature pertaining to myogenic stem cell populations that have potential for use in transplantation therapies to treat myopathies. Lastly, we will evaluate the main advances and drawbacks of using scaffolds and myogenic progenitor cells to produce engineered skeletal muscle tissue.

3. SKELETAL MUSCLE ORGANIZATION

The main function of skeletal muscle is contraction to perform voluntary mechanical work. To fulfill this role, skeletal muscles possess a highly specialized and well-defined structure (Figure 1) made up of thousands of cylindrical muscle fibers known as myofibers that extend the entire length of the muscle and attach to the bone through tendons. Myofibers are multinucleated single cells composed of an array of stacked myofibrils running the entire length of the cell. Myofibrils consist of thick and thin filaments organized into a contractile unit called a sarcomere (Figure 2). Shortening or contraction of the sarcomeres leads to contraction of myofibers, and subsequently the muscle as a whole.

Myofibers are surrounded by a basal lamina under which are located satellite cells, a population of quiescent muscle progenitor cells. Myofibers are also embedded in connective tissue with a rich network of blood vessels and nerves that are essential for providing nutrients

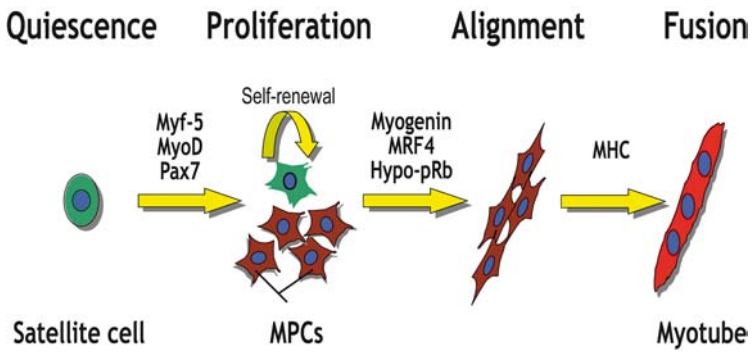

Figure 3. Satellite cells in muscle regeneration. Muscle regeneration is characterized by the activation of normally mitotically quiescent satellite cells and the differentiation of their daughter cells or myogenic precursor cells (MPCs). MPCs undergo multiple rounds of division that are characterized by the initial activation of Pax7 and two members of the myogenic regulatory factor family (Myf5 and MyoD), which up-regulate two other important MRFs (myogenin and MRF4) and block cell cycle progression via the hypo-phosphorylation of pRb. MPCs, align and fuse with pre-existing or new myofibers and undergo a terminal differentiation process whereby components such as myosin heavy chain (MHC) are expressed. Following activation, a number of MPCs return to a quiescent state to maintain a population of satellite cells.

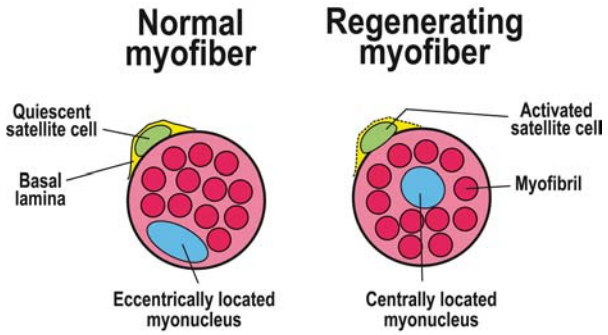

Figure 4. Cross-section of regenerating myofibers. The cross-section of newly regenerated muscle shows myofibers with centrally located nuclei that eventually move eccentrically to the cell membrane.

and stimuli for contraction. Skeletal muscle is organized into bundles of myofibers in the perimysium, which is surrounded by the epimysium.

4. Muscle Repair

Comprehending skeletal muscle repair is fundamental to understanding the complex biology of stem and progenitor cells that are necessary for designing effective tissue engineering strategies. Myofibers within adult skeletal muscle tissue are generally very stable, as demonstrated by the low turnover of myonuclei. Indeed, only 1-2% of the myonuclei are replaced every week under physiological conditions (2) compared to the high 6% turnover rate for red blood cells (3). However, muscles damaged by intensive exercise or trauma display a rapid, strong capacity to regenerate. Muscle regeneration is characterized by the activation of normally mitotically quiescent satellite cells and the differentiation of their daughter cells, which are called myogenic precursor cells (MPCs). When satellite cells become activated, they cross the basal lamina and migrate to sites where they are required. Their descendants, the MPCs, undergo multiple rounds of division prior to fusing with pre-existing or new myofibers and undergoing a terminal differentiation process (4) (Figure 3). These processes are characterized by an initial activation of Pax7 and two members of the myogenic regulatory factor family (MRFs), Myf5 and MyoD.

Figure 4 shows a cross-section of newly regenerated muscle containing myofibers with centrally located nuclei. In time, these myonuclei are pushed to the periphery of the cell membrane as the myofibers mature (5). The position of the nuclei within the myofiber is thus an indicator of muscle regeneration. Indeed, the number of myofibers with centrally located nuclei is indicative of the severity of the muscle damage. On the other hand, the amount of eccentrically located nuclei provides a measure of the potential of the myofiber to reacquire a mature, contractile phenotype.

5. Cellular Transplantation Therapy

Stem cells play a role in organ development during embryogenesis and contribute to tissue healing in

adults. They can thus be used as a source of cells for transplantation therapies and tissue engineering applications. Adult stem cells become activated when they are needed for maintaining tissue or repairing tissue damage, with some subsequently returning to a quiescent state to maintain the stem cell pool.

The potential differentiation capacity of stem cells is defined by four categories: (1) totipotent stem cells can give rise to the three germ layers of the embryo, the placenta (trophoblast) and primordial germ cells; (2) pluripotent stem cells are able to form all three germ layers; (3) multipotent stem cells differentiate into various cell types but only form one germ layer; and (4) progenitors or committed stem cells give rise only to one specific cell type.

Until recently, it was thought that satellite cells were the only source of myogenic stem cells. However, flow cytometry and cell culture methodologies have made it possible to isolate myogenic stem cells from other sources. A number of muscle-derived stem cells (MDSC) and non-MDSC have been characterized for their myogenic potential, and have been tested in transplantation experiments for their ability to integrate into muscle tissue.

5.1. Muscle-derived myogenic stem cells for transplantation therapy

5.1.1. Satellite cells

Satellite cells are the main source of adult myogenic stem cells in skeletal muscle. Primary myoblasts, which are cultured satellite cells isolated from skeletal muscle and injected into host muscle, are used in myoblast transplantation therapy (MTT). Partridge *et al.* set the standard for MTT by performing the first successful transplants in mice (6). Transplanted wild-type myoblasts generate dystrophin-positive myofibers in mdx mice, which contain a mutated dystrophin gene (7, 8). However, there appear to be limitations to the use of MTT in human applications as other animal models have not been as successful. Indeed, the poor ability of myoblasts to migrate to host tissues is a major drawback. For example, in monkeys, 25-65% of donor cell myofibers are integrated into the host tissue if the myoblasts are injected every 1 mm, while only 5-15% are integrated if they are injected every 2 mm (9). Pre-treating the myoblasts with metalloproteases (MMPs) is one way to circumvent this problem (10). MMPs modify the extracellular matrix (ECM), which significantly improves the migration and integration of donor myoblasts into host muscle tissue (10).

Another limitation of MTT is that microinjection causes an acute host immune response such that over 90% of donor myoblasts are eliminated within the first hour post-transplantation (11), with the majority being purged within minutes (12). This problem is difficult to surmount because the host immune response can act through three pathways composed of many different factors (13, 14). One pathway involves pre-existing "natural" host antibodies that induce a proteolytic cascade of the complement system, which leads to the rapid lysis of donor cells. The second involves direct interactions between host T cells and cell surface molecules expressed by donor myoblasts. The third involves the indirect activation of host T cells by host antigen-presenting cells that present donor antigens.

A further shortcoming of MTT is that a genetically defective myofiber may not be functionally restored by a donor nucleus. Consequently, there is no guarantee that the fusion of normal myoblasts with genetically defective myofibers will result in the expression of donor genes throughout the myofiber. Most intracellular proteins coded by a single nucleus in a myofiber remain confined to a small region known as the "nuclear domain" (15). The extent of the "nuclear domain" is mainly dependent on three factors. One is a physico-chemical restriction based on the ability of a protein to diffuse. The second is the propensity of some diffusible proteins to remain anchored to other components of the myofiber. The third is the fact that mRNA can only diffuse 100 μm from the nucleus that generates it (16, 17). This is evident from the experiments of Kinoshita *et al.*, who transplanted myoblasts from normal mice and from transgenic mice that over-express dystrophin (Tg-MDA) into dystrophin-deficient mdx mouse muscles (18). Four weeks post-transplantation, four times more dystrophin-positive fibers were observed in muscles injected with Tg-MDA than in those injected with normal myoblasts. In addition, the muscle fibers of the mdx mice injected with Tg-MDS myoblasts that displayed dystrophin were 1,500 μm long while those in the mdx mice injected with normal myoblasts were only 500 μm long. These experiments demonstrated that the transplantation of myoblasts highly over-expressing dystrophin (50-fold) only increase dystrophin expression in a restricted region of the membrane.

MTT has been used on dystrophin-deficient patients with some success. One study reported that only a few host myofibers expressed dystrophin after the injection of millions of myoblasts (19). Gussoni *et al.* (1997) reported encouraging results from six Duchenne muscular dystrophy patients who had been treated using MTT. Biopsies showed that over 10% of the original number of donor cells remained six months post-implantation, that half of the surviving donor cells had fused to the host myofibers, and that nearly 50% of these myofibers produced dystrophin (20). These results indicate that more work is required to develop an optimized MTT treatment strategy for human recipients.

5.1.2. Muscle-derived side population (SP) cells

Side population (SP) cells, another source of potential myogenic cells with hematopoietic characteristics for transplantation therapies, have also been isolated from skeletal muscles (21, 22). The isolation of this heterogeneous population of cells is based on fluorescence antigen cell sorting analysis (FACS) and on the unique property of these cells to actively exclude Hoechst 33342 dye due to cell surface multi-drug resistant proteins (23-28). Muscle SP cells (mSP) are capable, albeit less effectively than their bone marrow (BM) counterparts, of reconstituting all hematopoietic blood cell lineages when injected intra-venously into lethally-irradiated mice (26).

Importantly, mSP are capable of myogenic conversion *in vivo*. Indeed, when injected intravenously into female dystrophin-deficient mdx mice, 9% of the myofibers become dystrophin-positive while 3% of their myonuclei are Y-chromosome positive seventeen days post-injection (26).

5.1.3. Adult muscle-derived CD34 positive cells

The Sca-1^+CD34^+ heterogeneous cell fraction is another MDSC population that has been successfully used for cellular transplant therapy in mice (29-31). CD34 is expressed on myeloid progenitors and endothelial cells, and stem cell antigen-1 (Sca-1) is present on many stem cells (32-35). Successive pre-plating combined with FACS sorting using the membrane antigens Sca-1 and CD34 enriches the sub-population of myogenic cells (29). With the use of transgenic mice harbouring the LacZ reporter gene expressing beta-galactosidase (beta-gal) under the control of a muscle specific promoter the fate of donor cells when transplanted within a host could be tracked. Hence, when injected intra-arterially into mdx mice the Sca-1^+CD34^+ cells from LacZ reporter mice that activated their myogenic programme expressed beta-gal, that was detected by the addition of x-gal substrate eight weeks post-transplantation in all recipient muscle fibers. Interestingly, the migration and incorporation of these cells in the recipient musculature increased 12% following muscle injury, suggesting extensive damage causes them to become activated and enter the myogenic lineage (29).

5.1.4. Embryonic muscle-derived CD34-positive cells

Le Grand *et al.* demonstrated that a FACS-sorted $CD34^+FLK1^+$ cell population, which was isolated from embryonic mouse muscle, has myogenic potential (36). Their myogenic potential *in vivo* was demonstrated by transplanting $CD34^+Flk1^+$cells isolated from the muscle tissue of E17 mice expressing beta-gal under the control of a muscle specific promoter. Sixty percent of the total muscle length displayed beta-gal-positive myofibers, while 18% of the myofibers expressed dystrophin, indicating that they were able to integrate into the host muscle (36).

Unfortunately, bona fide satellite cells may be contaminated by a sub-population of satellite cells that also express CD34 (37, 38). This highlights the need for supplementary markers in order to successfully enrich MDSC populations (39, 40). Moreover, isolating cells from human embryos is fraught with ethical concerns.

5.2. Non-muscle-derived myogenic stem cells for transplantation therapy

5.2.1. Bone marrow-derived

Ferrari *et al.* first reported that non-resident myogenic stem cells have the potential to provide cells for muscle regeneration (41). In their experiments, BM transplantation of donor cells expressing beta-gal driven by a myogenic promoter were transplanted into irradiated immunodeficient mice (scid/bg). Beta-gal^+ cells were observed within the musculature of the recipients three weeks post-transplantation. However, the contribution of the donor cells to muscle regeneration was insignificant compared to that of endogenous satellite cells. The integration of BM-derived cells into the host musculature is enhanced by cardiotoxin, which is a non-physiological environment. Labarge *et al.*, in a more physiologically relevant milieu, demonstrated that BM-derived cells integrate into host muscles following exercise and that BM-derived cells expressing GFP make up as much as 3.5% of total muscle fibers following exercise (42).

A sub-population of BM-derived myogenic stem cells (BM-SP) has also been assessed for their regenerative myogenic capacity in mdx mice (26, 43). Some 100 to 500 BM-derived cells with myogenic activity were found in the SP fraction of each mouse (20). When BM-SP cells were injected into the tail vein of mdx mice, they were able to integrate into the host musculature, with 4% of the myofibers becoming dystrophin-positive within 12 weeks post-transplantation.

These observations recapitulate the results of a BM transplantation in a one-year-old Duchene muscular dystrophy patient (44). Thirteen years post-transplantation, the patient still possessed a small number of myofibers containing donor cell nuclei (44). While encouraging, these findings highlight the fact that BM-derived cells are inefficiently incorporated into host muscles, preventing their therapeutic use for the present (41, 45, 46).

5.2.2. Mesoangioblasts

Mesoangioblasts, another embryonically-derived cell type, have been reported to be a potential source of myogenic cells for therapeutic transplantation (47). These non-muscle-derived stem cells are isolated from the dorsal aorta of E9.5 mouse embryos using the explant method. They can be expanded in cell cultures for as many as 50 passages without demonstrating tumoral activity (48). A skeletal muscle wasting alpha-sarcoglycan (alpha-SG) null mouse model has been used to show that intra-arterial delivery of mesoangioblasts can provide cells for muscle regeneration (49). Twenty-four hours post-delivery, a third of the injected mesoangioblasts were detected in the muscles downstream from the injection site. However, if the cells were injected via the tail vein or intra-muscularly, fewer than a 3% of the donor cells were detected. The success of the protocol thus relies on the widespread distribution of donor cells through the capillary network, highlighting the importance of the delivery method. As these cells are embryonic in nature, their limited availability reduces their usefulness as a therapy.

6. ENGINEERED SKELETAL MUSCLE TISSUE

The engineering of skeletal muscle tissue for transplantation holds out substantial promise for the replacement of muscles that have been surgically removed or that have been severely damaged by major injuries or by skeletal muscle myopathy treatments. The current standard treatment involving the transfer of muscle tissue from other sites is not sufficient as it is associated with donor site weakness related to size and function deficits.

The three main components required for engineered muscle tissue are myogenic precursors, a matrix

for their efficient growth and the potential use of a scaffold material to support and enhance proper development and function. Some researchers use a "cells-in-gel" technique to produce three-dimensional (3D) constructs (50-52) (see Table 1). Basically, this method uses a suitable matrix in which myoblasts are mixed with a gel. The mixture is then transferred to a mold and allowed to set. After allowing the myoblasts a certain time to grow in the mold, differentiation media is added to promote myotube formation. The 3D muscle constructs must be maintained in a suitable matrix that supports the culture and growth of the cells. The matrix must be biocompatible and biodegradable for efficient integration into the host and must also have mechanical properties that enable the construct to support stretch and force production (50, 52-55). Moreover, it should provide a high surface area for cell-matrix interactions while maintaining a high rate of diffusion during *in vitro* culture (56, 57). This assists in maintaining the viability and function of the cells within the construct and avoiding necrosis.

6.1. Types of scaffolds

6.1.1. Natural scaffolds

Matrigel™ in combination with collagen is the most common biomaterial used as a matrix for cultivating 3D muscle cell constructs. Matrigel™ is an extract from the Engelbreth-Holm-Swarm mouse sarcoma that contains ECM proteins and undefined concentrations of growth factors that enhance myogenic differentiation (50, 58). Matrigel™ in combination with collagen allows myoblasts to differentiate *in vitro*, resulting in the formation of an artificial skeletal muscle containing parallel arrays of post-mitotic myofibers expressing sarcomeric contractile proteins (50). However, this artificial muscle lacks many normal morphological characteristics, including small diameter muscle fibers and a low density of myofibers, because of the high ECM content (50). Processed dermal sheep collagen discs have also been tested as a scaffold. Unfortunately, the implantation of this muscle construct in rats causes a massive infiltration of granulocytes and macrophages (59). While these matrices promote the myotube differentiation, they are not suitable for clinical use. However, they may be of great interest for experimental modeling (50).

The natural compound fibrin is another potential cell culture matrix that can be used to support muscle tissue growth. Fibrin is biocompatible and biodegradable and has a high binding affinity for biological surfaces (60, 61). In addition, fibrin is amenable to controlled alterations. The degradation rate can be modified by adding protease inhibitors such as aprotinin. Its porous structure can also be improved by incorporating thrombin at various concentrations to influence the diffusion of nutrition factors and hence cellular growth (62, 63). Beier *et al.* used fibrin as a 3D platform for delivering myoblasts. They harvested primary rat myoblasts from male rats, incorporated them into a 3D fibrin matrix and injected the mixture into the defective anterior gracilis muscles of female syngeneic rats (64). Interestingly, the myoblast-fibrin matrix enhanced the integration of the myoblasts within host muscle fibers without causing an inflammatory reaction (64). While the myoblast-fibrin injection technique can be used to restore skeletal muscle tissue locally, it cannot be adapted to pathologies such as muscular dystrophy, cachexia or muscle disuse, which require a more systemic approach.

6.1.2. Biocompatible polymeric scaffolds

A major problem in muscle tissue engineering is the lack of structural organization in the newly formed constructs. Muscle cells need to be oriented parallel to each other to ensure directed force production. The design of an appropriate scaffold able to support cell fusion and the formation of long continuous muscle fibers is one solution that has been developed to overcome this major shortfall. These scaffolds are made from a variety of biomaterials including biopolymers, synthetic polymers ceramics and metals. Engineered muscle fiber constructs fabricated in this way must be able to be surgically attached to host tissue and must conform to general tissue engineering requirements, including biocompatibility. Scaffold technology used to engineer muscle tissues must allow for the efficient transport of nutrients and for vascularization, which is essential for resident cell viability. Oxygen diffusion, nutrient delivery, waste removal, protein transfer and cell migration are critical factors that are governed by the porosity and permeability properties of the scaffold.

Degrapol®, an electrospun degradable polyesterurethane membrane, is a promising scaffold for skeletal muscle tissue engineering in that it can be used to engineer soft tissues because of its elastic properties. Degrapol® has been shown to promote favorable cell/scaffold interactions with C2C12 and L6 cells (55). Cells adhere, proliferate and differentiate into multinucleated myotubes expressing myogenic markers (55, 65). Degrapol® also promotes C2C12 cell adhesion and myotube alignment along the scaffold fibers. It also degrades within 180 days, which is another important feature (55). Despite these promising results, there are no reports in the literature to date on the post-transplantation performance of Degrapol® constructs.

Other muscle tissue scaffold technologies make use of microfibrous polymers composed of poly-L-lactic acid (PLLA), a classic degradable polyester, or polyglycolic acid (PGA). It has been shown that human skeletal muscle cells can easily differentiate into multinucleated myofibers on a PLLA scaffold coated with ECM proteins. More importantly, ECM-coated PLLA scaffolds enable cells to form functional muscle by directing the organization of myofibers into a parallel orientation (66). PGA meshes have been used to engineer skeletal muscle tissue *in vivo*. Differentiated neonatal rat myoblast constructs implanted in rat peritoneal cavities display well-vascularized 3D structures with the ability to generate neo-muscle-like tissue six weeks post-transplantation (67, 68).

6.1.3. Bio-inspired adhesive polymeric scaffolds

Over the years, a variety of strategies have been implemented to manipulate fabricated muscle tissue with the functions and characteristics of real muscle. These strategies include both chemical and mechanical

Table 1. Scaffolds used in muscle tissue engineering

Material	Cell Response	Natural (N)/ Synthetic (S)	Properties	Physiological Application	Ref.
3D skeletal muscle construct Myoblast/fibroblast co-cultures	-Cell proliferation -Cell differentiation -Myotube formation -Low muscle force -Low excitability	N	Does not require pre-existing scaffold	-*In vitro* experiments only	(75, 77)
Collagen disc	-Infiltration of granulocytes and macrophages *in vivo*	N	Biodegradable	Transplantation unsuccessful	(59)
Fibrin/myoblast	-Cell differentiation *in vivo* -Myoblasts integrate into host fiber	N	-Biocompatible -Biodegradable -Does not require pre-existing scaffold	-Transplantation successful -Only suitable for restoring skeletal muscle locally	(64)
3D skeletal muscle Collagen/Matrigel	-Cell proliferation -Cell differentiation -Myotube formation -Small diameter muscle fibers -Low myofiber density -Excessive ECM	N	-Degradable-Directs myofiber orientation *in vitro*	-*In vitro* experiments only	(50, 52)
Degrapol® coated with Matrigel or Collagen	-Cell proliferation -Cell differentiation -Myotube formation	S/N	-Elastic -Favorable cell/scaffold interaction -Degradable -Directs myofiber orientation	-*In vitro* experiments only	(55, 65)
Co-polymer PLLA/PGA co-cultured with myoblast, fibroblasts and endothelial cells	-Cell proliferation -Cell differentiation -Myotube formation	S/N	-Degradable -Directs myofiber orientation	-Transplantation successful	(87)
-PLLA -PGA -Alginate hydrogel -Hyaluronic acid hydrogel	-Cell proliferation -Cell differentiation -Myotube formation	S	-Degradable -Directs myofiber orientation	-Transplantation successful	(66-68)

perturbations that have gone a long way to improving the performance of specific muscle tissue traits.

The use of integrins is another strategy to enhance the efficient organization of engineered skeletal muscle. Integrins are a widely expressed family of heterodimeric transmembrane receptors that bind to adhesive motifs in various ECM proteins, including fibronectin, vitronectin, laminin and collagen (69). Adhesive cells utilize ECM proteins not only for attachment and migration but also for exchanging signals that direct cell functions to prevent apoptosis, maintain cell cycle progression, and determine tissue-specific phenotypes (70-72). In particular, $alpha_5beta_1$ integrin control proliferation and differentiation of myoblasts (72). The identification of recognition sequences that mediate integrin-mediated adhesion (73) has stimulated the development of bio-inspired adhesive surfaces to which myoblasts can adhere. In one study, alkanethiol self-assembled monolayers making up a highly ordered nano-sized molecular surface were used to analyze the effects of surface chemistry on myoblast proliferation and differentiation. Coating the monolayers with fibronectin revealed that surface chemistry modulates myoblast proliferation and differentiation via $alpha_5beta_1$ integrin binding. Interestingly, blocking $beta_1$ but not $beta_3$ integrin inhibits differentiation, which indicates that specific integrins are involved in myoblast differentiation (74). Immobilizing molecules using surface chemistry may provide important insights into cell-matrix interactions and the management of specific cell behaviors influenced by these interactions.

6.2. Current challenges

6.2.1. Force generation

A shortcoming of tissue-engineered muscles is the lack of sufficient force generation. In most studies forces generated by fabricated constructs fall are 2-8% lower than those generated by adult mammalian skeletal muscle (75-77). However, a recent study has shown that the force generated by engineered muscle can be significantly improved by adding insulin-like growth factor 1 (IGF-1), a well-known skeletal muscle anabolic factor (51, 52). The improvement of muscle force by IGF-1 may be the result of an increase in myofiber size (40%), in contractile protein synthesis and enhanced sarcomeric organization. On the other hand, atorvastatin, a cholesterol lowering statin known to induce muscle weakness, causes myofiber atrophy and decreases muscle force (52).

Mechanical and electrical stimulation are used to improve the organization and function of engineered skeletal muscle. During myogenesis, mechanical stimulation influences the expression of genes and proteins as well as the parallel alignment of myotubes (50, 78-81). Engineered muscle constructs submitted to stretch and relaxation cycles by a mechanical cell stimulator display a high muscle fiber-to-ECM ratio and increased elasticity (50). Without mechanical stimulation, the *in vitro* construct exhibits morphological and functional characteristics that differ from those of native skeletal muscle. Indeed, engineered skeletal muscle that has not undergone mechanical stimulation has a higher ECM content and a lower myofiber density, and does not undergo complete maturation (76, 82).

Rat myoblasts stimulated with different electrical impulse patterns produce variable levels of myosin heavy chain expression and myotube formation (83, 84). In addition, electrically stimulating muscles enhances the expression of vascular endothelial growth factor (VEGF), which in turn stimulates the migration and proliferation of endothelial cells required during angiogenesis (85).

6.2.2. Vascularization

Chemical treatments have been successfully used to enhance vascularization, another important feature of muscles. Frequently, the interior of scaffold constructs, unlike the periphery, does not support viable tissue due to poor diffusion. One strategy to circumvent this problem is to deliver the angiogenic protein-encoding gene in order to stimulate vascularization. Injecting myoblasts transduced with a retrovirus carrying murine angiogenic vascular endothelial growth factor (VEGF) cDNA into mouse leg muscle showed that continuous VEGF delivery had deleterious effects resulting in the formation of hemangiomas containing localized networks of vascular channels (86). Recently, Langer's laboratory induced the formation of blood vessel networks in engineered skeletal muscle tissue (87) by seeding a porous biodegradable polymer scaffold made of PLLA and PGA with myoblasts, embryonic fibroblasts and endothelial cells (87, 88). The myoblasts differentiated and formed myotubes. The tissue construct also produced a large number of blood vessels from endothelial cells stimulated by VEGF secreted by the embryonic fibroblasts. When this engineered construct was implanted in mice and rats, the long, thick donor myotubes aligned with the host tissue fibers. In addition, donor vessels containing red blood cells formed along the host myotubes, indicating that the vessels had anastomosed with the recipient vasculature. However, using such constructs for clinical application requires further studies on their biocompatibility, handling, viability and contractility.

7. CONCLUDING REMARKS

Muscular myopathies continue to defy treatment strategies. While much work remains to be done to develop a successful myogenic-stem-cell transplantation protocol, myogenic stem cells remain the best strategy for treating muscular myopathies. For transplantation therapies to succeed, major obstacles must be overcome, including developing a suitable method for delivering the stem cells and ensuring their survival in the host, as well as coming to a better understanding of the mechanisms involved in the modulation their myogenic commitment and differentiation. Engineered skeletal muscle tissue at an early stage of development is another powerful muscle tissue replacement strategy that, if successfully developed, will make it possible to deliver a large number of viable, properly differentiated myogenic stem cells with an enhanced capacity to integrate into recipient muscles. For now, engineered skeletal muscle is in the embryonic stage of development and requires significant investigation before being introduced into the clinical setting.

8. ACKNOWLEDGMENTS

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9. REFERENCES

1. Blau, H.M., T.R. Brazelton, J.M. Weimann: The evolving concept of a stem cell: entity or function? *Cell*, 105, 829-41 (2001)

2. Schmalbruch, H., D.M. Lewis: Dynamics of nuclei of muscle fibers and connective tissue cells in normal and denervated rat muscles. *Muscle Nerve*, 23, 617-26 (2000)

3. Berlin, N.I..: The life span of the red cell. *Prog Clin Biol Res*, 11, 21-31 (1976)

4. Bischoff, R.: Chemotaxis of skeletal muscle satellite cells. *Dev Dyn*, 208, 505-15 (1997)

5. Pophal S, J.J Evans, P.E. Mozdziak. Myonuclear apoptosis occurs during early posthatch starvation. *Comp Biochem Physiol B Biochem Mol Biol*, 135, 677-81 (2003)

6. Partridge, T.A., M. Grounds, J.C Sloper: Evidence of fusion between host and donor myoblasts in skeletal muscle grafts. *Nature*, 273, 306-8 (1978)

7. Morgan, J.E., D.J. Watt, J.C Sloper, T.A. Partridge: Partial correction of an inherited biochemical defect of skeletal muscle by grafts of normal muscle precursor cells. *J Neurol Sci*, 86, 137-47 (1988)

8. Partridge, T.A., J.E. Morgan, G.R. Coulton, E.P. Hoffman, L.M. Kunkel: Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature*, 337, 176-9 (1989)

9. Skuk, D., M. M. Goulet, B. Roy, J.P. Tremblay: Efficacy of myoblast transplantation in nonhuman primates following simple intramuscular cell injections: toward defining strategies applicable to humans. *Exp Neurol*, 175, 112-26 (2002)

10. Torrente, Y., E. El Fahime, N.J. Caron, N. Bresolin, J.P. Tremblay: Intramuscular migration of myoblasts transplanted after muscle pretreatment with metalloproteinases. *Cell Transplant*, 9, 539-49 (2000)

11. Beauchamp, J.R., C.N Pagel, T.A. Partridge: A dual-marker system for quantitative studies of myoblast transplantation in the mouse. *Transplantation*, 63, 1794-7 (1997)

12. Hodgetts, S.I., M.W. Beilharz, A.A. Scalzo, M.D. Grounds: Why do cultured transplanted myoblasts die *in vivo*? DNA quantification shows enhanced survival of

donor male myoblasts in host mice depleted of CD4+ and CD8+ cells or Nk1.1+ cells. *Cell Transplant*, 9, 489-502 (2000)

13. Smythe, G.M., S.I. Hodgetts, M.D. Grounds: Immunobiology and the future of myoblast transfer therapy. Mol Ther, 1, 304-13 (2000)

14. Smythe, G.M., S.I. Hodgetts, M.D. Grounds: Problems and solutions in myoblast transfer therapy. *J Cell Mol Med*, 5, 33-47 (2001)

15. Pavlath, G.K., K. Rich, S.G. Webster, H.M. Blau: Localization of muscle gene products in nuclear domains. *Nature*, 337, 570-3 (1989)

16. Hall, Z.W., E. Ralston: Nuclear domains in muscle cells. *Cell*, 59, 771-2 (1989)

17. Ralston, E., Z.W. Hall: Restricted distribution of mRNA produced from a single nucleus in hybrid myotubes. *J Cell Biol*, 119, 1063-8 (1992)

18. Kinoshita, I., J.T. Vilquin, I. Asselin, J. Chamberlain, J.P. Tremblay: Transplantation of myoblasts from a transgenic mouse overexpressing dystrophin prduced only a relatively small increase of dystrophin-positive membrane. *Muscle Nerve*, 21, 91-103 (1998)

19. Mendell, J.R., J.T. Kissel, A.A. Amato, W. King, L. Signore, T.W. Prior, Z. Sahenk, S. Benson, P.E. McAndrew, R. Rice *et al.*: Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *N Engl J Med*, 333, 832-8 (1995)

20. Gussoni, E., H.M. Blau, L.M. Kunkel: The fate of individual myoblasts after transplantation into muscles of DMD patients. *Nat Med*, 3, 970-7 (1997)

21. Kawada, H., M. Ogawa M: Hematopoietic progenitors and stem cells in murine muscle. *Blood Cells Mol Dis*, 27, 605-9 (2001)

22. Kawada, H., M. Ogawa: Bone marrow origin of hematopoietic progenitors and stem cells in murine muscle. *Blood*, 98, 2008-13 (2001)

23. Zhou, S., J.D. Schuetz, K.D. Bunting, A.M. Colapietro, J. Sampath, J.J. Morris, I. Lagutina, G.C Grosveld, M. Osawa, H. Nakauchi, B.P. Sorrentino: The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med*, 7, 1028-34 (2001)

24. Zhou, S., J.J. Morris, Y. Barnes, L. Lan, J.D. Schuetz, B.P. Sorrentino: Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells *in vivo*. *Proc Natl Acad Sci U S A*, 99, 12339-44 (2002)

25. Goodell, M.A., K. Brose, G. Paradis, A.S. Conner,R.C. Mulligan: Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. *J Exp Med*, 183, 1797-806 (1996)

26. Gussoni, E., Y. Soneoka, C.D. Strickland, E.A. Buzney, M.K. Khan, A.F. Flint, L.M. Kunkel, R.C. Mulligan: Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*, 401, 390-4 (1999)

27. Asakura, A., P. Seale, A. Girgis-Gabardo, M.A. Rudnicki: Myogenic specification of side population cells in skeletal muscle. *J Cell Biol*, 159, 123-34 (2002)

28. Jackson, K.A., T. Mi, M.A. Goodell: Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci U S A*, 96, 14482-6 (1999)

29. Torrente, Y., J.P. Tremblay, F. Pisati, M. Belicchi, B. Rossi, M. Sironi, F. Fortunato, M. El Fahime, M.G. D'Angelo, N.J. Caron, G. Constantin, D. Paulin, G. Scarlato, N. Bresolin: Intraarterial injection of muscle-derived CD34(+)Sca-1(+) stem cells restores dystrophin in mdx mice. *J Cell Biol*, 152, 335-48 (2001)

30. Jankowski, R.J.,B.M. Deasy, B. Cao, C. Gates, J. Huard: The role of CD34 expression and cellular fusion in the regeneration capacity of myogenic progenitor cells. *J Cell Sci*, 115, 4361-74 (2002)

31. Qu-Petersen, Z., B. Deasy, R. Jankowski, M. Ikezawa, J. Cummins, R. Pruchnic, J. Mytinger, B. Cao, C. Gates, A. Wernig, J. Huard: Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. *J Cell Biol*, 157, 851-64 (2002)

32. Krause, D.S., T, Ito, M.J. Fackler, O.M. Smith, M.I. Collector, S.J. Sharkis, W.S. May: Characterization of murine CD34, a marker for hematopoietic progenitor and stem cells. *Blood*, 84, 691-701 (1994)

33. Fennie, C., J. Cheng, D. Dowbenko, P. Young, L.A. Lasky: CD34+ endothelial cell lines derived from murine yolk sac induce the proliferation and differentiation of yolk sac CD34+ hematopoietic progenitors. *Blood*, 86, 4454-67 (1995)

34. Morel, F., S.J. Szilvassy, M. Travis, B. Chen, A. Galy: Primitive hematopoietic cells in murine bone marrow express the CD34 antigen. *Blood*, 88, 3774-84 (1996)

35. Scime, A., G. Grenier, M.S. Huh, M.A. Gillespie, L. Bevilacqua, M.E. Harper, M.A. Rudnicki: Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. *Cell Metab*, 2, 283-95 (2005)

36. Le Grand, F., G. Auda-Boucher, D. Levitsky, T. Rouaud, J. Fontaine-Perus, M.F. Gardahaut MF: Endothelial cells within embryonic skeletal muscles: a potential source of myogenic progenitors. *Exp Cell Res*, 301, 232-41 (2004)

37. Beauchamp, J.R., L. Heslop, D.S. Yu, S. Tajbakhsh, R.G. Kelly, A. Wernig, M.E. Buckingham, T.A Partridge, P.S. Zammit: Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. *J Cell Biol*, 151, 1221-34 (2000)

38. Kuang, S., S.B. Charge, P. Seale, M. Huh, M.A. Rudnicki: Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. *J Cell Biol*, 172, 103-13 (2006)

39. Beauchamp, J.R., J.E. Morgan, C.N. Pagel, T.A. Partridge: Dynamics of myoblast transplantation reveal a discrete minority of precursors with stem cell-like properties as the myogenic source. *J Cell Biol*, 144, 1113-22 (1999)

40. Lee, J.Y., Z. Qu-Petersen, B. Cao, S. Kimura, R. Jankowski, J. Cummins, A. Usas, C. Gates, P. Robbins, A. Wernig, J. Huard: Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing. *J Cell Biol*, 150, 1085-100 (2000)

41. Ferrari, G., G. Cusella-De Angelis, M. Coletta, E. Paolucci, A. Stornaiuolo, G. Cossu, F. Mavilio: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science*, 279, 1528-30 (1998)

42. LaBarge, M.A., H.M. Blau HM: Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell*, 111, 589-601 (2002)

43. Bittner, R.E., C. Schofer, K. Weipoltshammer, S. Ivanova, B. Streubel, E. Hauser, M. Freilinger, H. Hoger, A. Elbe-Burger, F. Wachtler: Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. *Anat Embryol (Berl)*, 199, 391-6 (1999)

44. Gussoni, E., R.R. Bennett, K.R. Muskiewicz, T. Meyerrose, J.A. Nolta, I. Gilgoff, J. Stein, Y.M. Chan, H.G. Lidov, C.G. Bonnemann, A. Von Moers, G.E. Morris, J.T. Den Dunnen, J.S. Chamberlain, L.M. Kunkel, K. Weinberg: Long-term persistence of donor nuclei in a Duchenne muscular dystrophy patient receiving bone marrow transplantation. *J Clin Invest*, 110, 807-14 (2002)

45. Heslop, L., J.R. Beauchamp, S. Tajbakhsh, M.E. Buckingham, T.A. Partridge, P.S. Zammit: Transplanted primary neonatal myoblasts can give rise to functional satellite cells as identified using the Myf5nlacZl+ mouse. *Gene Ther*, 8, 778-83 (2001)

46. Wernig, A., M. Zweyer, A. Irintchev: Function of skeletal muscle tissue formed after myoblast transplantation into irradiated mouse muscles. *J Physiol*, 522 Pt 2, 333-45 (2000)

47. De Angelis, L., L. Berghella, M. Coletta, L. Lattanzi, M. Zanchi, M.G. Cusella-De Angelis, C. Ponzetto, G. Cossu: Skeletal myogenic progenitors originating from embryonic dorsal aorta coexpress endothelial and myogenic markers and contribute to postnatal muscle growth and regeneration. *J Cell Biol*, 147, 869-78 (1999)

48. Minasi, M.G., M. Riminucci, L. De Angelis, U. Borello, B. Berarducci, A. Innocenzi, A. Caprioli, D. Sirabella, M. Baiocchi, R. De Maria, R. Boratto, T. Jaffredo, V. Broccoli, P. Bianco, G. Cossu: The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development*, 129, 2773-83 (2002)

49. Sampaolesi, M., Y. Torrente, A. Innocenzi, R. Tonlorenzi, G. D'Antona, M.A. Pellegrino, R. Barresi, N. Bresolin, M.G. De Angelis, K.P. Campbell, R. Bottinelli, G. Cossu: Cell therapy of alpha-sarcoglycan null dystrophic mice through intra-arterial delivery of mesoangioblasts. *Science*, 301, 487-92 (2003)

50. Powell, C.A., B.L. Smiley, J. Mills, H.H. Vandenburgh HH: Mechanical stimulation improves tissue-engineered human skeletal muscle. *Am J Physiol Cell Physiol*, 283, C1557-65 (2002)

51. Shansky, J., B. Creswick, P. Lee, X. Wang, H. Vandenburgh: Paracrine release of insulin-like growth factor 1 from a bioengineered tissue stimulates skeletal muscle growth *in vitro*. *Tissue Eng*, 12, 1833-41 (2006)

52. Vandenburgh, H., J. Shansky, F. Benesch-Lee, V. Barbata, J. Reid, L. Thorrez, R. Valentini, G. Crawford: Drug-screening platform based on the contractility of tissue-engineered muscle. *Muscle Nerve* (2008)

53. Mikos, A.G., G. Sarakinos, S.M. Leite, J.P. Vacanti, R. Langer: Laminated three-dimensional biodegradable foams for use in tissue engineering. *Biomaterials*, 14, 323-30 (1993)

54. Freed, L.E., G. Vunjak-Novakovic, R.J. Biron, D.B. Eagles, D.C. Lesnoy, S.K. Barlow, R. Langer: Biodegradable polymer scaffolds for tissue engineering. *Biotechnology (N Y)*, 12, 689-93 (1994)

55. Riboldi, S.A., M. Sampaolesi, P. Neuenschwander, G. Cossu, S. Mantero: Electrospun degradable polyesterurethane membranes: potential scaffolds for skeletal muscle tissue engineering. *Biomaterials*, 26, 4606-15 (2005)

56. Rebel, J.M., W.I. De Boer, C.D. Thijssen, M. Vermey, E.C. Zwarthoff, T.H. Van der Kwast: An *in vitro* model of urothelial regeneration: effects of growth factors and extracellular matrix proteins. *J Pathol*, 173, 283-91 (1994)

57. Ye, Q., G. Zund, P. Benedikt, S. Jockenhoevel, S.P. Hoerstrup, S. Sakyama, J.
A. Hubbell, M. Turina M: Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. *Eur J Cardiothorac Surg*, 17, 587-91 (2000)

58. Dusterhoft, S., D. Pette D: Satellite cells from slow rat muscle express slow myosin under appropriate culture conditions. *Differentiation*, 53, 25-33 (1993)

59. van Wachem, P.B., L.A. Brouwer, M.J. van Luyn: Absence of muscle regeneration after implantation of a collagen matrix seeded with myoblasts. *Biomaterials*, 20, 419-26 (1999)

60. Bach, A.D., H. Bannasch, T.J. Galla, K.M. Bittner, G.B. Stark: Fibrin glue as matrix for cultured autologous urothelial cells in urethral reconstruction. *Tissue Eng*, 7, 45-53 (2001)

61. Juhasz, I., G.F. Murphy, H.C. Yan, M. Herlyn, S.M. Albelda: Regulation of extracellular matrix proteins and integrin cell substratum adhesion receptors on epithelium during cutaneous human wound healing *in vivo*. *Am J Pathol*, 143, 1458-69 (1993)

62. Clark, R.A., J.M. Lanigan, P. DellaPelle, E. Manseau, H.F. Dvorak, R.B. Colvin: Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization. *J Invest Dermatol*, 79, 264-9 (1982)

63. Albelda, S.M., C.A. Buck: Integrins and other cell adhesion molecules. *Faseb J*, 4, 2868-80 (1990)

64. Beier, J.P., J. Stern-Straeter, V.T. Foerster, U. Kneser, G.B. Stark, A.D. Bach: Tissue engineering of injectable muscle: three-dimensional myoblast-fibrin injection in the syngeneic rat animal model. *Plast Reconstr Surg*, 118, 1113-21; discussion 1122-4 (2006)

65. Riboldi, S.A., N. Sadr, L. Pigini, P. Neuenschwander, M. Simonet, P. Mognol, M. Sampaolesi, G. Cossu, S. Mantero: Skeletal myogenesis on highly orientated microfibrous polyesterurethane scaffolds. *J Biomed Mater Res A*, 84, 1094-101 (2008)

66. Cronin, E.M., F.A. Thurmond, R. Bassel-Duby, R.S. Williams, W.E. Wright, K.D. Nelson, H.R. Garner: Protein-coated poly(L-lactic acid) fibers provide a substrate for differentiation of human skeletal muscle cells. *J Biomed Mater Res A*, 69, 373-81 (2004)

67. Saxena, A.K., J. Marler, M. Benvenuto, G.H. Willital, J.P. Vacanti: Skeletal muscle tissue engineering using isolated myoblasts on synthetic biodegradable polymers: preliminary studies. *Tissue Eng*, 5, 525-32 (1999)

68. Saxena, A.K., G.H. Willital, J.P. Vacanti: Vascularized three-dimensional skeletal muscle tissue-engineering. *Biomed Mater Eng*, 11, 275-81 (2001)

69. Hynes, R.O.: Integrins: bidirectional, allosteric signaling machines. *Cell*, 110, 673-87 (2002)

70. Menko, A.S., D. Boettiger: Occupation of the extracellular matrix receptor, integrin, is a control point for myogenic differentiation. *Cell*, 51, 51-7 (1987)

71. Zhu, X., M. Ohtsubo, R.M. Bohmer, J.M. Roberts, R.K. Assoian: Adhesion-dependent cell cycle progression linked to the expression of cyclin D1, activation of cyclin E-cdk2, and phosphorylation of the retinoblastoma protein. *J Cell Biol*, 133, 391-403 (1996)

72. Garcia, A.J., M.D. Vega, D. Boettiger: Modulation of cell proliferation and differentiation through substrate-dependent changes in fibronectin conformation. *Mol Biol Cell*, 10, 785-98 (1999)

73. Ruoslahti, E., M.D. Pierschbacher: Arg-Gly-Asp: a versatile cell recognition signal. *Cell*, 44, 517-8 (1986)

74. Lan, M.A., C.A. Gersbach, K.E. Michael, B.G. Keselowsky, A.J. Garcia: Myoblast proliferation and differentiation on fibronectin-coated self assembled monolayers presenting different surface chemistries. *Biomaterials*, 26, 4523-31 (2005)

75. Dennis, R.G., P.E Kosnik 2nd: Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered *in vitro*. *In vitro Cell Dev Biol Anim*, 36, 327-35 (2000)

76. Kosnik, P.E., J.A. Faulkner, R.G. Dennis: Functional development of engineered skeletal muscle from adult and neonatal rats. *Tissue Eng*, 7, 573-84 (2001)

77. Dennis, R.G., P.E Kosnik 2nd, M.E. Gilbert, J.A. Faulkner: Excitability and contractility of skeletal muscle engineered from primary cultures and cell lines. *Am J Physiol Cell Physiol*, 280, C288-95 (2001)

78. Goldspink, D.F., V.M. Cox, S.K. Smith, L.A. Eaves, N.J. Osbaldeston, D.M. Lee, D. Mantle: Muscle growth in response to mechanical stimuli. *Am J Physiol*, 268, E288-97 (1995)

79. Goldspink, D.F.: Exercise-related changes in protein turnover in mammalian striated muscle. *J Exp Biol*, 160, 127-48 (1991)

80. Goldspink, G., A. Scutt, J. Martindale, T. Jaenicke, L. Turay, G.F. Gerlach: Stretch and force generation induce rapid hypertrophy and myosin isoform gene switching in adult skeletal muscle. *Biochem Soc Trans*, 19, 368-73 (1991)

81. Vandenburgh, H.H.: Dynamic mechanical orientation of skeletal myofibers *in vitro*. *Dev Biol*, 93, 438-43 (1982)

82. Vandenburgh, H.H.: Functional assessment and tissue design of skeletal muscle. *Ann N Y Acad Sci*, 961, 201-2 (2002)

83. Dusterhoft, S., D. Pette D: Effects of electrically induced contractile activity on cultured embryonic chick breast muscle cells. *Differentiation*, 44, 178-84 (1990)

84. Naumann, K., D. Pette D: Effects of chronic stimulation with different impulse patterns on the expression of myosin isoforms in rat myotube cultures. *Differentiation*, 55, 203-11 (1994)

85. Nagasaka, M., M. Kohzuki, T. Fujii, S. Kanno, T. Kawamura, H. Onodera, Y. Itoyama, M. Ichie, Y. Sato: Effect of low-voltage electrical stimulation on angiogenic growth factors in ischaemic rat skeletal muscle. *Clin Exp Pharmacol Physiol*, 33, 623-7 (2006)

86. Springer, M.L., A.S. Chen, P.E. Kraft, M. Bednarski, H.M. Blau: VEGF gene delivery to muscle: potential role for vasculogenesis in adults. *Mol Cell*, 2, 549-58 (1998)

87. Levenberg, S., 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 R: Engineering vascularized skeletal muscle tissue. *Nat Biotechnol*, 23, 879-84 (2005)

88. Levenberg, S., N.F. Huang, E. Lavik, A.B. Rogers, J. Itskovitz-Eldor, R. Langer: Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. *Proc Natl Acad Sci U S A*, 100, 12741-6 (2003)

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