Review: Whole Skeletal Muscle Transplantation: Mechanisms Responsible for Functional Deficits

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One aspect of tissue engineering of skeletal muscle involves the transposition and transplantation of whole muscles to treat muscles damaged by injury or disease. The transposition of whole muscles has been used for many decades, but since 1970, the development of techniques for microneurovascular repair has allowed the transplantation of large muscles. Transposition and transplantation of muscles invariably result in structural and functional deficits. The deficits are of the greatest magnitude during the first month, and then a gradual recovery results in the stabilization of structural and functional variables between 90 and 120 days. In stabilized vascularized grafts ranging from 1 to 3 g in rats to 90 g in dogs, the major deficits are ~25% decrease in muscle mass and in most grafts ~40% decrease in maximum force. The decrease in power is more complex because it depends on both the average shortening force and the velocity of shortening. As a consequence, the deficit in maximum power may be either greater or less than the deficit in maximum force. Tenotomy and repair are the major factors responsible for the deficits.

Although the data are limited, skeletal muscle grafts appear to respond to training stimuli in a manner no different from that of control muscles. The training stimuli include traditional methods of endurance and strength training, as well as chronic electrical stimulation. Transposed and transplanted muscles develop sufficient force and power to function effectively to: maintain posture; move limbs; sustain the patency of sphincters; partially restore symmetry in the face; or serve as, or drive, assist devices in parallel or in series with the heart.

Key words: transposition • microneurovascular repair • tenotomy and repair • muscle atrophy • motor unit number • mean motor unit maximum force.

INTRODUCTION

For specific applications to skeletal muscle, "tissue engineering" is defined as the rational modification of the structure and function of skeletal muscles by manipulation of molecules, cells, or constituent structures of the whole tissue. Skeletal muscles may be "engineered" by utilizing the natural adaptive, or restorative, capacities, by introduction of gene sequences into the genome of the animal, or by transplantation of cells, whole tissues, or biologic substitutes (modified from University of Michigan, Bioengineering Program, August, 1992). In animals, the presence of some 660 skeletal muscles, which comprise 40% of the total body mass, provides the potential for a wide variety of muscle injuries and defects and, conversely, many possibilities for the utilization of skeletal muscles in tissue repair. This provides the basis for one aspect of the burgeoning field of tissue engineering, the transposition and transplantation of whole skeletal muscles.

Studitsky[31] was the first to transplant skeletal muscle tissue successfully. In rats, gastrocnemius muscles were minced and packed back into the same site, and a substantial mass of muscle fibers regenerated. Subsequently, the free whole muscle grafting technique developed and proved to be more effective in terms of the mass of muscle that regenerated than the minced muscle grafts. For whole muscle grafts, a series of investigations, first by Carlson and Gutmann, and later by Carlson and Faulkner, clarified the processes of ischemic necrosis, revascularization, and fiber degeneration, regeneration, innervation, and differentiation, as well as the functional recovery.[5] Free whole muscle transplantation permitted the transplantation of muscles up to 6 g in mass, but in larger grafts spontaneous revascularization was not adequate and grafts were infiltrated with large masses of fat and connective tissue.[10] Clinically, the small, free, whole muscle grafts provided an operative technique for the treatment of partial facial paralysis and urinary and anal incontinence.[15]

Muscles larger than 6 g may be transposed as a myofascial flap with the vasculature intact, or transplanted with microneurovascular repair. The myofascial flap, first described by Golovine in 1898,[18] has been adapted for the treatment of a wide variety of defects. The requirement is for a muscle with sufficient mass, and consequently adequate force and power, to perform the tasks expected of the defective, or absent, muscle in the recipient site. The architecture of muscle does not change following transposition, or transplantation with neurovascular repair.[20] Consequently, the pennation of the donor muscle should be matched to that of the muscle in the recipient site. Because fiber types demonstrate a high

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degree of plasticity in response to changed innervation and loading,[13] the matching of the proportion of fiber types in the donor muscle to those of the muscle in the recipient site is not critical, or even necessary. The muscle must have a single, major, neurovascular pedicle. In this procedure, depending on the requirements of the operation, one or both tendons are severed, but the vascular pedicle remains intact. The muscle is then transposed to the new site and the tendon(s) is sutured to the tendon stump(s), or an appropriate insertion. Muscles may be transposed with the nerve intact, or, if a new innervation is required, the nerve may be sectioned and repaired.

For restoration of mimetic function following partial facial paralysis[14] and oropharyngeal reconstruction,[24] the temporalis flap has proved effective. For injuries to muscles in the head, neck, upper arm, forearm, and chest wall, the muscle of choice has been the latissimus dorsi (LTD) muscle.[25] Following transposition of the LTD muscle, adduction and internal rotation of the arm are not impaired significantly, apparently because the force and power generated by the teres major and pectoralis muscles provide an adequate substitute.[23] The broad, flat LTD muscle is extremely versatile either in its natural configuration for reconstruction of the pectoralis major muscle, or rolled and sutured to substitute for the biceps or triceps muscles. Uses for the LTD muscle continue to evolve. To assist a failing heart, the LTD muscle has been transposed into the thoracic cavity and wrapped around the heart, or alternatively wrapped around a pump in series with the heart.[27]

In 1970, the application of microsurgical techniques to the repair of nerves and vasculature permitted the transplantation of large whole muscles regardless of their mass and opened up a whole new area of skeletal muscle transplantation.[32] As with the transposition of muscles, a single neurovascular pedicle must be present. In addition, the neurovascular pedicle must be suitable for the repair by microsurgical techniques. The criteria necessary for a successful outcome are a sufficient muscle mass to provide the adequate force and power and the presence of a nerve to provide an adequate number of motoneurons to innervate the muscle fibers in specific motor units. Reinnervation of a more, or less, normal number of motor units is a necessary condition for the establishment of the volitional patterns of recruitment and subsequent muscle contractions that underlie everyday movements.

Transplantation of whole muscles with neurovascular repair has enabled portions of, or whole, gracilis muscles to be transplanted into the sites of the malfunctioning zygomaticus major, and finger-flexor muscles; and LTD muscles to be transplanted into the site of the traumatized quadriceps muscle.[14,15] For patients with anal incontinence, gracilis and gluteus maximus muscles have been looped around the anus to serve as a neosphincter.[1]

In spite of the potential that vascularized grafts offer for the restoration of structure and function in denervated, injured, and diseased muscles, the grafts do display structural and functional deficits. A stabilized graft is defined as a graft that has ceased to demonstrate any further improvement with time following the operative procedure.[19,21] For stabilized grafts, the deficits are not significantly different for transposed muscles and muscles transplanted with neurovascular repair.[4,21] Throughout this review, these vascularized grafts will be designated as transposed, or transplanted, muscles, or collectively, as vascularized grafts. On occasion, the status of the nerve, intact, or repaired, will be of importance.

The purpose of this review is to describe the structural and functional deficits observed in stabilized transposed and transplanted muscles, clarify the underlying mechanisms responsible for the deficits, and outline potential methods of rehabilitation to overcome the deficits. The review will close with a brief summary of the clinical implications of whole skeletal muscle transposition and transplantation in tissue engineering.

STRUCTURAL DEFICITS

Although the major structural deficit in vascularized grafts is muscle atrophy, atrophy is not a result of significant muscle fiber necrosis and degeneration.[19] Less than 10% of the fibers show any morphological indications of degeneration. The time course of the changes in structural and functional deficits following grafting of vascularized muscles with nerves intact and with nerves repaired has been described most completely for the 9 g rectus femoris (RFM) muscle in the rabbit (Figure 1). Within the first 7 days after grafting, the mass of both types of vascularized grafts decreases to 77% of the control value. By 14 days, the mass of the nerve-intact grafts returns to the control value, whereas the mass of nerve-repaired grafts decreased further to 56%. By 90 days, the masses of both nerve-intact and nerve-repaired vascularized grafts stabilize at 75% of the value for control muscles (Figure 1A).

During the first 30 days after grafting, the mean single fiber cross-sectional area (CSAs) for nerve-intact and nerve-repaired vascularized grafts differ greatly, but are not different from each other after 60 days (Figure 1B). For the nerve-repaired vascularized grafts, denervation atrophy is responsible for the dramatic early loss in mass and mean single fiber CSA of.[19,21] During the first 30 days after grafting, the decrease in muscle mass is independent of the decrease in the single fiber CSAs (Figure 1A and B). At 15 days after grafting, the 15% decrease in muscle mass of nerve-intact vascularized grafts occurs with no change in single fiber CSA, and for the nerve-repaired vascularized grafts the 33% loss in muscle mass was accompanied by a 50% loss in single fiber CSA (Figure 1A and B). In contrast, in control muscles and stabilized grafts, the value for single fiber CSAs is highly predictive of the muscle mass. When single fiber CSAs have stabilized between 90 days and 120 days, both types of grafts display an approximate 25% loss in mass and a slightly larger (38%) loss in single fiber CSA.

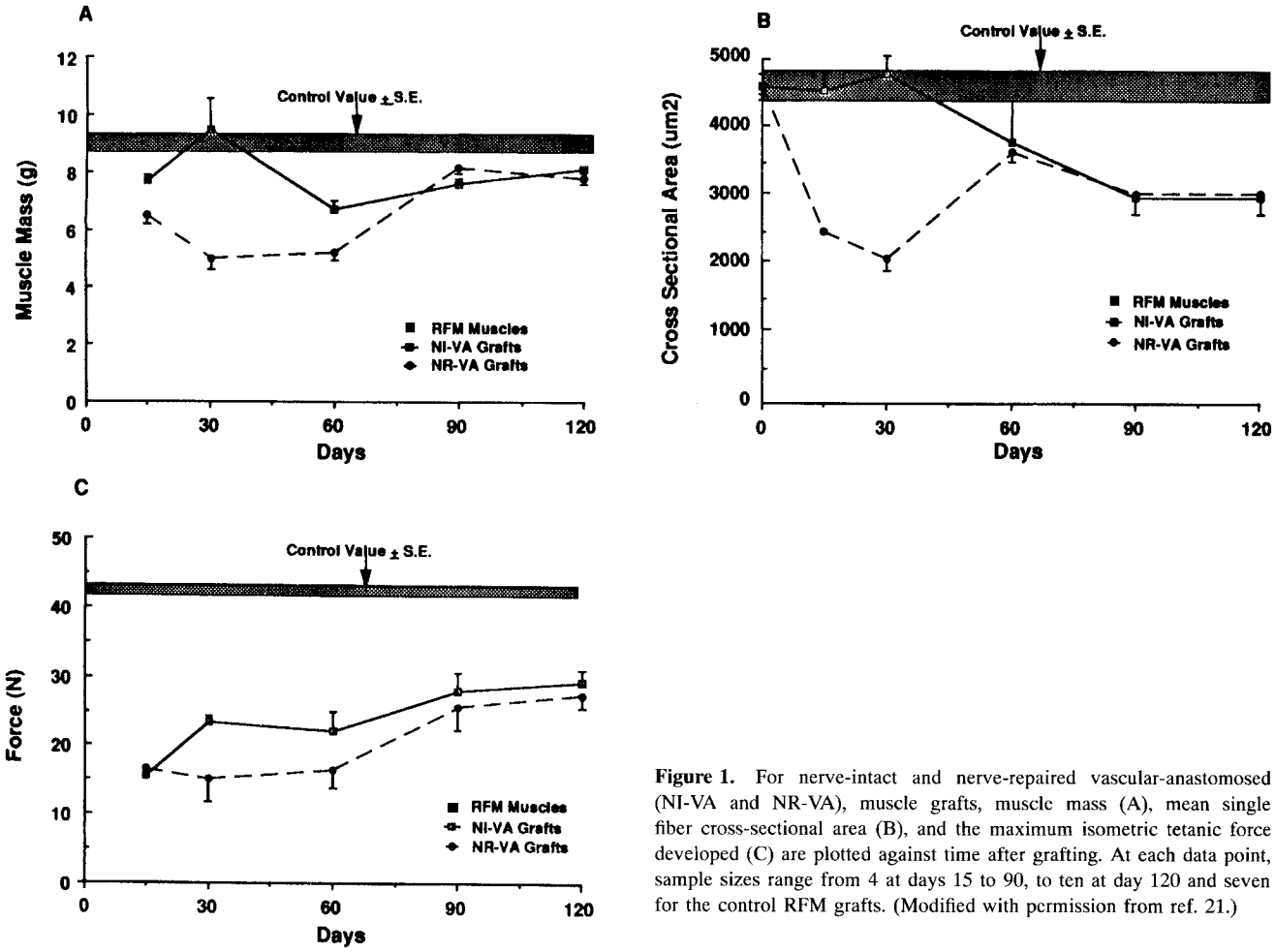

Figure 1. For nerve-intact and nerve-repaired vascular-anastomosed (NI-VA and NR-VA), muscle grafts, muscle mass (A), mean single fiber cross-sectional area (B), and the maximum isometric tetanic force developed (C) are plotted against time after grafting. At each data point, sample sizes range from 4 at days 15 to 90, to ten at day 120 and seven for the control RFM grafts. (Modified with permission from ref. 21.)

The total muscle fiber CSA of grafts and control muscles may be calculated from the algorithm: muscle mass (kg)/[fiber length (meters) $\times$ 1060 (kg/m^3)].[20] Division of the total muscle fiber CSA by the mean single fiber CSA provides an estimate of the total number of fibers in a graft or a control muscle (Figure 2). Although the methods of estimation are indirect, the estimates of the total number of fibers in nerve-intact and nerve-repaired vascularized grafts are not different from those of control donor muscles.[19,20]

FUNCTIONAL DEFICITS

The best measures of the functional recovery achieved by a transposed or transplanted muscle are the maximum isometric tetanic force and the maximum power. The maximum force provides the best prediction of the ability of the graft to maintain posture, or maintain the patency of a sphincter, whereas maximum power provides the best prediction of the ability of the graft to move limbs, or fluids.[25] The maximum force is a function of the total muscle fiber CSA. The division of the maximum force by the total muscle fiber CSA provides a value of the maximum specific force (kN/m^2). Division of power (watts) by mass provides a normalized value of the maximum power (W/kg). The normalized values for force and power permit the evaluation of the functional recovery of the graft independent of the effects of muscle atrophy.

For nerve-intact and nerve-repaired vascularized grafts, only limited data are available on the changes in maximum force over time (Figure 1C), and no data are available on the changes in maximum power. In both types of grafts, the maximum forces decrease to 40% at 15 days and then gradually recover to 60% of the control values at 120 days. The grafts with nerves intact show a more rapid recovery at day 30 and day 60. For both types of vascularized grafts, the values for force development at 90 days are not different from those at 120 days (Figure 1C).

When stabilized, the maximum forces are not different for nerve-intact and nerve-repaired vascularized grafts.[4,20] With vasculature intact or repaired, a number of different donor muscles have been grafted into a wide variety of recipient sites in different species (rats, rabbits, and dogs). The donor muscles have differed in mass from 1 g to 80 g, and in muscle architecture from bipennate to parallel-fibered. In spite of the diversity of donor muscles, sites, and species, the deficit in mean maximum force varies only from 30% for the 1 g MGN grafts in rats[33] to between 40% and 50% for the remainder of the grafts. In contrast for any given study, compared with the maximum force

developed by control muscles, the recovery of maximum force for single vascularized grafts is highly variable, with a range from 25% to 100%.

When the maximum force is normalized for total muscle fiber CSA, the deficits in maximum specific force are 15% for grafts in rats[33] and 25% for those in rabbits[4] and dogs.[26] The LTD muscle transplanted into the RFM muscle site in rabbits is the only graft with a maximum specific force not different from the control value.[20] Surprisingly, after tenotomy and repair, LTD grafts in rats have a deficit in maximum specific force of 16%.[25]

Because of the greater difficulty in making measurements of power, particularly on large muscles, much less data are available on the maximum power generated by grafts than for the maximum force developed.[25] Furthermore, since maximum power is a function of both the average force during shortening and the velocity of shortening, the deficits for maximum power are more complex than those for maximum force. Compared with the deficits for maximum force, the deficits for maximum power may be of either greater,[33] or lesser[25] magnitude. The differences for the deficits in force and power are a result of differences in the deficits for shortening versus isometric force and in the change between the graft and the control muscle for optimum velocity for the development of power. Compared with the control muscle, the optimum velocity for power may either increase, or decrease. In rats, MGN muscles grafted into the same site with neurovascular repair have a deficit in maximum power of 50%, whereas for the LTD muscles exposed to tenotomy and repair, the deficit is 30%. The deficits for normalized maximum powers were 45% and 0%, respectively.

MECHANISMS UNDERLYING DEFICITS IN STABILIZED GRAFTS

Three operative procedures have the potential, singly or in concert, to cause the 25% decrease in the muscle mass of stabilized grafts: (1) the anastomoses of the blood vessels; (2) the repair of the nerves; and (3) the sectioning and repair of the tendons. No difference is observed between the mass, or maximum force of grafts made with anastomoses of the vasculature and those made with vasculature intact.[4,21] Furthermore, when normalized per 100 g of viable muscle, the maximum blood flow for vascularized grafts is not different from that of control muscles.[3] We conclude that the repair of arteries and veins does not have any effect on the structural, or functional, properties of vascularized grafts.

Under conditions in which the vasculature and tendons remained intact, the sectioning and subsequent repair of the nerve results in a 15% deficit in maximum force, whereas simply implanting the nerve in the muscle produces a 38% deficit.[17] The assessments were made 6 months after the operation, and neither procedure produced any change in muscle mass. When the whole muscle deficit in the maximum force of vascularized grafts is 40%, the major deficit arises from a decrease of 25% in the number of motor units with only a 15% deficit in the maximum force of single motor units (Figure 3). Following

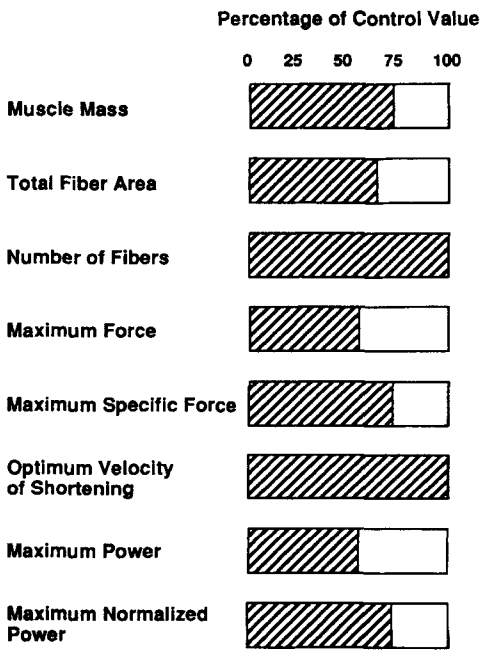

Figure 2. The structural and functional deficits in nerve-reparied vascularized grafts of rectus femoris muscles in rabbits expressed as a percentage of the values for the control rectus femoris muscle. (The data are a summary for data from refs. 4, 19, and 33.)

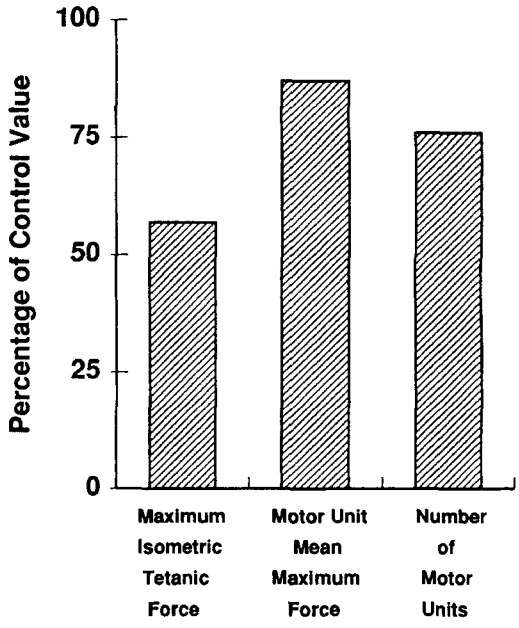

Figure 3. Deficits in the maximum isometric tetanic force developed by whole nerve-repaired vascularized grafts of medial gastrocnemius (MGN) muscles, the mean maximum force developed by motor units in the grafts, and the number of motor units present in grafts. Values are expressed as a percentage of the appropriate value for control MGN muscles. (The bar grafts are based on data from ref. 12.)

either procedure, the deficit in maximum force appears to arise from incomplete innervation with some of the motor nerves failing to establish a functioning motor unit (Figure 3). If such is the case, nerve-repair is more effective than nerve-implantation in the number of motor units established.

The reason is not clear for the equally large deficit in maximum force and presumably motor unit number for nerve-intact and nerve-repaired vascularized grafts. When both types of grafts have stabilized, the deficit in maximum force associated with tenotomy and tendon repair with the neurovascular pedicle intact is not different from that observed for a muscle transplanted with neurovascular repair.[4,21] The conclusion is that tenotomy and repair is the predominant factor contributing to muscle atrophy and the force deficit. The mechanism by which tenotomy and repair initiates these structural and functional impairments in muscle grafts is unknown.[21]

In vascularized grafts, the deficit in specific force is 30% when calculated from the division of maximum force by total muscle fiber CSA. The dry mass–wet mass ratio of stabilized grafts is not different from that of control muscle.[4] Compared with control muscles, stabilized grafts have a three-fold increase in connective tissue.[4,21] Although the magnitude of the increase is highly significant, connective tissue concentration constitutes less than 2% of the muscle mass.[4] Consequently, the increase in connective tissue has a negligible effect of ~5% on the total muscle fiber CSA.[4] In vascularized grafts, after correction of the total muscle fiber CSA for the increase in connective tissue and the decrease in total protein content, the deficit in specific force is approximately 25% (Figure 2).

The cause of the unexplained portion of the deficit in specific force is not immediately evident.[4] One possibility is that, although the total number of fibers in the vascularized grafts is not different from the number in the control donor muscle, some proportion of the fibers in the grafts might remain denervated. Denervated fibers are known to have impaired contractile function, and this could explain the deficit in specific force.

In vascularized grafts, both the number of motor units and the mean maximum force of motor units are decreased (Figure 3). The decrease in the mean maximum force of the motor units in vascularized grafts results from the combined effects of a 17% increase in the number of fibers per motor unit and a decrease of 30% in the mean maximum force per fiber. For fibers within motor units, the mean maximum force per fiber decreases because of the decrease in the mean single fiber CSA (Figure 1). The assumption is that the specific force of fibers within motor units in grafts is not different from that of fibers in control muscles. Based on the number of motor units in grafts and the number of fibers per motor unit, the estimate of the number of innervated fibers is less for vascularized grafts than for control muscles. We conclude that the overall results are consistent with the hypothesis that a population of denervated fibers exists in vascularized grafts.

The deficit in the maximum power is a complex interplay between the effect of muscle atrophy on the average force during shortening and the optimum velocity of shortening for maximum power. Grafts tended to show small, but insignificant, increases in optimum velocity of shortening. With the limited data available, whether this is an adaptive increase based on faster isoforms of myosin heavy chains,[29] or merely fortuitous, is not clear.

The deficits in mass, force, and power of stabilized vascular grafts (Figure 2) are similar for 1 g medial gastrocnemius (MGN) muscles in rats,[33] 3 g LTD muscles in rats,[25] 9 g RFM muscles in rabbits,[4,19] 12 g LTD muscles in rabbits,[20] and 80 g gracilis muscles in dogs.[8,26] Consequently, for vascularized grafts, the mass of muscle transposed, or transplanted, has no bearing on the magnitude of the structural, or functional, deficits. Presumably, comparable deficits would be present in even larger muscles transposed and transplanted to treat muscle defects in human beings, but exact data are not available.[16]

RESOLUTION OF DEFICITS IN MUSCLE GRAFTS

In some circumstances the anticipated structural and functional deficits in muscle grafts may be resolved by the transposition, or transplantation, of a larger mass of muscle. The postoperative atrophy and loss in specific force and normalized power then simply reduce the structural and functional properties to the appropriate values. This technique is often used in grafting muscles into the face to correct partial facial paralysis.[22] If too large or too powerful a mass of muscle remains when the graft has stabilized, a second operation is required to trim the graft down to the appropriate mass and function.[22] This method of resolving the deficits has limited application because most often a greater mass of muscle than is required is not available.

The structural and functional deficits observed for vascularized grafts could be reduced, or even eliminated, by appropriate strength, or endurance, conditioning of the graft. Small (150 mg) free whole-muscle grafts in rats respond to both chronic low frequency (10 Hz) stimulation[6] and ablation of synergistic muscles[9] in a manner not different from that of control muscles. Consequently, the possibility of applying stimulation protocols developed on control muscles to vascularized grafts would appear reasonable. In spite of the potential for effective conditioning of vascularized grafts, no data are available on the response of vascularized grafts in small mammals to either strength, or endurance, conditioning. A few anecdotal reports of volitional strength conditioning by patients are consistent with the data on free grafts in rats that grafts respond and adapt to physical conditioning in a manner not different from that reported for control muscles.[13]

Chronic low-frequency stimulation protocols convert fibers in control muscles and free grafts to essentially 100% type I fibers with some decrease in muscle mass

and single fiber CSA.[6,28] Effective protocols of short bursts of high-frequency stimulation that convert muscles to high proportions of type II fibers have been more difficult to develop.[28] At present, a satisfactory protocol has not been developed that would ensure the conversion of fibers in vascularized grafts to type II, or fast fibers. Such a conversion would be important for applications requiring high maximum, or sustained power, since fast muscles have ~three-fold greater capacity for the development of power than slow muscles.[2]

Chronic low-frequency electrical stimulation has been used to convert fast fibers to slow fibers prior to transposing latissimus dorsi muscles in cardiomyoplasty.[27] Although such a conversion increases the endurance capacity of the muscle, maximum force is reduced by the atrophy of fibers and the decrease in total muscle cross-sectional area. In addition, the decrease in optimum velocity for power significantly decreases the maximum and sustained power. In contrast to the need for power, when a transposed muscle is serving as a cardiac assist pump, only sustained force is required for a muscle to serve as a neosphincter. Therefore, conversion from fast- to slow-fiber characteristics with chronic low-frequency stimulation would be advisable before or after graciloplasty to reconstruct the anal sphincter.[1]

The evidence that free whole muscle grafts respond to the removal of synergistic muscles with a hypertropic response suggests that vascularized grafts will adapt positively to strength conditioning programs.[9] A conversion from type I to type II fibers coupled with an increased muscle mass could significantly increase the maximum force and to an even greater extent the maximum power of grafts. The removal of synergistic muscles does not provide a viable training protocol to increase the mass and force development of vascularized grafts.

CLINICAL IMPLICATIONS FOR TISSUE ENGINEERING WITH WHOLE SKELETAL MUSCLES

In spite of the deficits in muscle mass, maximum force, and maximum power (Figure 2), transposed and transplanted skeletal muscles are able to develop sufficient force to function effectively in the maintenance of posture and in sustaining the patency of urinary and anal sphincters. In addition, the maximum power of bundles of fiber segments of slow and fast fibers from muscles of human beings generate ~120 W/kg and ~40 W/kg respectively, and even more importantly, have the ability to sustain ~4 W/kg.[11] This level of sustained power enables transposed and transplanted muscles in human beings to serve as a cardiac assist pump,[27] in the movement of limbs,[14,15] and in the development of facial symmetry and facial expression.[14,15]

For tissue engineering of skeletal muscle, future directions for whole skeletal muscle transposition and transplantation appear to be in the resolution of the mechanisms responsible for the structural and functional deficits. Clarification of the molecular and cellular events contributing to these deficits would provide a rational foundation for their treatment and subsequent reduction or elimination. In the interim, development of suitable protocols of electrical stimulation with appropriate frequency for the development of endurance and loading for the induction of muscle fiber hypertrophy could do much to reduce the atrophy and weakness present in stabilized grafts.

Gene therapy constitutes another form of tissue engineering for skeletal muscle. In the genetic engineering of skeletal muscle, gene therapy has been used to treat specific muscle diseases, such as Duchenne muscular dystrophy, rather than deficits arising from the transposition, or transplantation, of whole muscles. In the treatment of mdx mice, a full-length dystrophin transgenes with a creatine kinase promotor was injected into the mouse embryos and the progeny overexpressed dystrophin in skeletal and cardiac muscle fibers.[7] The overexpression of dystrophin completely corrected the structural and functional impairments usually associated with dystrophic skeletal muscles, including the diaphragm.[30] The restoration of normal values for force and power in transgenic mdx mice supports the possibility of gene therapy for patients with Duchenne muscular dystrophy, but treatment of patients with dystrophy will require the development of a truncated dystrophin gene and a suitable viral vector to provide a safer mode of administration.

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