Long-Term Aneurally Regenerated Skeletal Myofibers into Heart: How Long They Could Survive and Grow by Phasic Daily FES?

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Abstract

Tissue or Cell Cardiac Bioassists (Cardiac-Bio-Assists) are biological approaches to remedy to cardiac progressive insufficiency based on autologous tissue or cell transplantation. Some of the work-hypotheses are in pre-clinical evaluation (implants of embryonic myocardiocytes or Skeletal Muscle Ventricle, SMV), others are under preliminary (Dynamic Aortomyoplasty, and heart implants of myoblasts derived from skeletal muscle satellite cells or potential cardiomyogenic stem cells from bone marrow) or advanced clinical testing (Dynamic Cardiomyoplasty).

When injected into myocardium satellite cell-derived myoblasts undergo cell death, but some proliferate, differentiate and fuse to myotubes, which then increase in size in spite of never being re-innervated by functional motoneurons. We will here discus data of long-term viability of aneurally regenerated muscle in experimental animals and in humans. Furthermore, we will provide new evidence that functional electrical stimulation (FES) allows growth of aneurally regenerated myofibers to almost normal values.

If artificial synapses could be designed and developed (to restrict activation to satellite cell derived myofibers, and avoid potentially arrhythmogenic excitability of the surrounding myocardiocytes) FES of the myofibers could add systolic assistance to benefits of satellite cell cardiac bio-assists.

Key words: aneural myogenesis, autologous tissue or cell transplants, Cardiac-Bio-Assists, cellular cardiomyoplasty, functional electrical stimulation, long-term denervation, muscle regeneration, myoblasts, skeletal muscle satellite cell.

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Morbility and morbidity of congestive cardiac insufficiency, differently than others cardiac diseases, do not decrease, in spite of significant progress of pharmacological treatments, due to increased longevity of the population. Cardiac failure is the most frequent cause of death in Europe, with 500.000 new cases every year and 50% mortality after five years. Cardiac insufficiency heavily limits autonomy and social relations of the patients, which experience an early functional aging. Therefore, two are the goals of any therapy: improve quality of life and increase survival [15]. Alternatives to current pharmacological treatments for advanced heart failure are mandatory due to clinical needs.

Cardiac transplant is the therapy of election when cardiac failure become pharmacologically intractable, but all over the developed world (nothing to say, the situation in the underdeveloped countries) the number of heart transplants has reached a limit set by the availability of donor organs. Though admission to transplant list is more and more selective, 25% of patients still die while awaiting a heart. In the near or far future xenotransplants could solve this problem, but even being optimists on their development and reliability, they would carry the risk of anthropozoonotic viral infections [96].

Ventriculoplasty is attracting interest of many cardiac surgeons, but because it involves discarding up to 300 g of not necessarily diseased myocardium, it is difficult to view it as other than an extreme remedy to severely dilated Cardiomyopathies. It currently carries high preoperative mortality and an adequate assessment of its benefits awaits the outcome of properly conducted trials [8, 59]. Mechanical artificial hearts will continue to be used mainly as a bridge to transplant. Even if the problems of hemocompatibility and infection were solved, the need of an external power supply might pose an unacceptable psychological challenge to the patient over the longer time. As suggested in a recent overview, the way to long lasting clinical use of Mechanical Circula-

tory Support is a long and winding road [58]. Against this background, Cardiac-Bio-Assists, surgical biological alternatives to cardiac assists, based on use of autologous transplants of skeletal muscle tissue or myogenic cells onto or into myocardium remains an attractive goal, worth being pursued strenuously [80].

Cardiac-Bio-Assists

To date the clinical use of skeletal muscle assistance has been confined to cardiomyoplasty and aortomyoplasty. In these procedures the LD muscle is wrapped around existing structures, the ventricles of the heart in cardiomyoplasty, or the ascending or descending aorta in aortomyoplasty. Experience with aortomyoplasty is limited as yet in terms of the number of patients (still less than 20 worldwide) and the duration of follow-up [11]. Cardiomyoplasty, on the other hand, has been carried out in over 1000 patients worldwide since its introduction in 1985, and there is now a substantial body of follow-up data [26]. Procedures like cardiomyoplasty and aortomyoplasty have the considerable advantage that they do not place new nonendothelial surfaces in contact with the circulating blood. However, they suffer from the limitation that the geometry of the pump is dictated by the size and shape of the existing organs. A grossly hypertrophied heart may be too large to be wrapped effectively by the patient's LD muscle. The branching of major vessels makes it difficult to wrap an appreciable length of the ascending aorta. The small lumen of the descending aorta restricts the stroke volume that can be achieved by compressing it. However, just as important as these anatomical considerations are the loading conditions imposed on the muscle wrap. In such a situation, the muscle is constrained to operate far from the peak of its power curve. The power available for assisting the heart can be harnessed much more effectively if the grafted muscle is formed into a separate auxiliary pump or skeletal muscle ventricle. The skeletal muscle ventricle is not constrained by existing geometry but is limited only by the size, shape, and fiber orientation of the muscle that is available. Subject only to these limitations, cavity volume, wall thickness, and direction of wrap can, in principle, be optimized to provide the maximum pumping performance. Set against this functional potential is the need to ensure that the introduction of such a device into the patient's circulation does not cause thrombus formation with the risks of obstruction of flow through the device and embolism to vital organs [80].

Other Options are Related to Cellular Cardiac Bioassistance

Cellular cardiac bioassistance

Cell-Cardiac-Bio-Assist (CCBA) is a biological approach to remedy to cardiac failure based on autologous or heterologous cell transplantation.

When confronted with the problem of heart damage after infarction, replacement of myocytes is the ideal scenario [76]. Cardiomyocyte replacement could be achieved by stimulating proliferation of endogenous mature cardiomyocytes or stem cells, or by implanting exogenous donor cardiomyocytes possibly derived from stem cells [3, 47, 50, 66].

The enhancement of cardiomyocyte proliferation after damage must be rapid if the subject is to survive any acute insult that disrupts heart function. In case of chronic damage, the proliferation might need to be sustained slowly but continually to replace myocytes during the course of the disease. A concurrent revascularization must also keep pace to secure repair and prevention of major scar tissue formation. The new myocardium must integrate into the surviving myocardial wall if it could function as the tissue it replaces. Even small areas of imperfectly integrated tissues are likely to severely alter electrical conduction and syncytial contraction of the heart, with long-term life-threatening consequences. All of this must occur while the heart continues to supply blood throughout the organism. Because traditionally mature cardiomyocytes are considered to be incapable of cell replication, the donor cardiomyocytes to date have largely been of embryonic/fetal origin. Some experiments in animal models report successful engraftment and maturation of embryonic cardiomyocytes in normal and injured hearts [56]. Other studies show that most of the donor cardiomyocytes (engrafted into mature rat hearts after infarction) retained their embryonic phenotype and did not form junctions with mature heart cells by 4 weeks.

Although neonatal donor cells could form junctions with host myocardium, there was massive initial death of donor cells and at later times the grafts were often isolated by scar tissue. This problem is a direct result of the inflammation and scarring after infarction. Cardiomyocyte transplantation therapy could be more effectively developed to address functional improvement in myopathic heart diseases [99]. Alternatively, possible engineering of cardiac grafts within three-dimensional scaffolds or aggregates might provide a superior source of donor cells for repopulation of the infarcted heart. A more attractive approach is the use of donor cardiomyocytes, but mixed results have been obtained with donor fetal/embryonic/neonatal cardiomyocytes and the use of such donor cells in humans raises major ethical issues. The use of skeletal muscle cells to repair damaged heart muscle has had some success, and experiments are ongoing.

Skeletal Myoblasts

Attempts to use autologous skeletal muscle to repair damaged hearts has been attempted by relocating wraps of whole sheets of the latissimus dorsi muscle to supplement myocardial function [65]. This process, called cardiomyoplasty, was pioneered in the 1980s [87] and has been applied clinically with varied success. Alternatively, transplantation of isolated cultured myoblasts has been used to try to restore muscle function in hearts both ex-

perimentally and in the clinical situation [45]. This requires the myoblasts to be extracted from skeletal muscle, expanded in tissue culture, and then usually injected into the heart muscle. Delivery via the circulation is an alternative to convey donor myoblasts to the whole heart. In all cases one of the critical goals [75, 90], electromechanical coupling through gap junction communication between the engrafted skeletal myoblasts (or the derived myotube/myofiber) and the surrounding cardiac myocytes, is out of discussion when properly tested [74]. Recent studies have improved the survival of transplanted myoblasts by heat shock pretreatment, modified their immunoprotection, and confirmed that the benefits of skeletal myoblast transfer are additive with those of conventional therapies, such as angiotensin-converting enzyme inhibition [27, 71]. Autologous myoblast transplantation is reported in human patients, with promising improvements in cardiac function [92]. Bioheart, Inc. announced in September 2002 FDA authorization to initiate clinical studies of its investigational product, MyoCell(TM), for heart muscle repair in the United States. The Myocell(TM) and MyoCath(TM) products are currently part of clinical trials on going in Europe. In May 2001, Bioheart, Inc. initiated the first and only clinical trial in the world for catheter-based delivery of cultured autologous myoblasts, MyoCell(TM), intended as a therapy for regenerating damaged muscle in heart failure patients. In May 2002 investigators announced that one-year results from the first MyoCell(TM) treated patient revealed a left ventricular ejection fraction (LVEF) increase from a baseline of 39% in May 2001 to 56%. Bioheart has received authorization from European authorities to enroll 10 additional patients in the Bioheart sponsored multi-center European MyoCell(TM) trial. Bioheart has received authorization from the Food and Drug Administration (FDA) to enroll 15 MyoCell(TM) patients in the United States in a Phase I study adjunct to bypass (Scott R. Bromley, personal communication).

When injected in myocardium the majority of satellite cell-derived myoblasts undergoes cell death, but some proliferate, differentiate and fuse to myotubes, which then increase in size in spite of never being reinnervated by functional motoneurons.

We will here discus data of long-term viability of aneurally regenerated muscle in experimental animals and in humans. Furthermore, we will provide new evidence that functional electrical stimulation (FES) allows growth of aneurally regenerated myofibers to almost normal values. If artificial synapses could be designed and developed (to restrict activation to satellite cell derived myofibers, and avoid potentially arrhythmogenic excitability of the surrounding myocardiocytes) FES of the myofibers could add systolic assistance to benefits of satellite cell cardiac bio-assists.

Effects of long-term permanent denervation on skeletal muscle

Let me first define long-term muscle denervation and its effects. We will use the term long-term denervation to identify a long-lasting period of post denervation muscle atrophy accompanied by degeneration (lipodystrophy and fibrosis) and non-compensatory reactions (myofiber generation and regeneration) of muscle tissue. In the time scale of rodent life (3–4 years), these events start one month after permanent denervation and last all animal life. There are large interspecies differences in rate of atrophy, extent of myogenic reactions and tissue degeneration, which are known in part, being possibly related to metabolic and life span diversity (see below).

In recent years "muscle plasticity" concept was used to stress that myofibers modulate gene expression to accommodate their profile of structural and enzymatic protein isoforms to changing functional demands [2, 39, 69, 82, 91]. In the life of myofibers denervation is a major event, which imposes a larger series of consequences than recognized in the "old good times" [7, 42]. Therefore I extend the concept to cover all the muscle reactions to denervation, as outlined in Table 1.

Atrophy. Denervation of myofibers early induces muscle atrophy, which is a reduction of tissue mass (usually expressed as wet weight) that could be attained by reduction of each myofiber (pure atrophy that could be better indicated as *hypotrophy*) and/or loss of myofibers by cell death (*hypoplasia*). We would use "*severe atrophy*", when hypotrophy reaches 90% reduction in myofiber size (cross section myofiber area or minimum diameter). It is accompanied by disarray of the sarcomeres up to disappearance. Chains of myonuclei mark severe atrophy, but hypernucleosis is also present in early new myofibers (myotubes). To distinguish between these two events an anti-embryonic myosin stain is necessary and sufficient. Myonuclear loss and myofiber death could be early events following denervation (see below), while long anucleated longitudinal sections with several clumped myonuclei along the myofiber are markers of long-lasting severe atrophy [95]. Disruption of molecular mechanisms tethering nuclei to actin cytoskeleton [77, 88] are possibly responsible of these nuclear clumpings, beside to lead to Emery-Dreifuss muscular dystrophy [6, 14]. On the other hand, muscle clumps remind nuclei of the synaptic endplate. Instead of being evidence of myofibers degeneration, myonuclei clumps may be due to overexpression of gene programs related to the long-lasting ability of myofibers to survive being ready to be reinnervated even far from their original end-plate.

Myofiber death

Cell death and its mechanisms in rat skeletal muscle undergoing post-denervation atrophy are now known in details [12]. Clear morphological manifestations of muscle cell death, with ultrastructural characteristics

Table 1. Long-term denervation muscle plasticity.

MUSCLE PLASTICITY
FIBER TYPING (ISOGENES' SWITCHING)
FAST FIBERS ⇄ SLOW FIBERS
TROPHISM & DEGENERATION
HYPOTROPHY — EUTROPHY — HYPERTROPHY
↓ SEVERE ATROPHY, MYOFIBER DEATH, LIPO/FIBRO/DYSTROPHY — ↓ HYPERPLASIA
MYOFIBER DEATH AND REGENERATION
Increased (?) Function → GENERATIVE MYOGENESIS
Cell Death → REGENERATIVE MYOGENESIS

very similar if not identical to those considered as nuclear and cytoplasmic markers of apoptosis are found. With increasing time of denervation, progressive destabilization of the differentiated phenotype of muscle cells is observed: spatial disorganization of myofibrils and formation of myofibril-free zones. These changes initially appears in subsarcolemmal areas near myonuclei, and by 4-month denervation they are spread throughout the sarcoplasm. Dead muscle fibers are usually surrounded by a folded intact basal lamina; they have an intact sarcolemma and highly condensed chromatin and sarcoplasm. Folds of the basal lamina around the dead cells result from significant shrinkage of cell volume. Macrophages are occasionally found in close proximity to dead myocytes.

Manifestations of inflammation are absent in the denervated tissue. A search for molecular markers of apoptosis, nuclear DNA fragmentation by the TUNEL method, reveals the presence of multiple DNA fragments in cell nuclei in only a very small number of cell nuclei in 2- and 4-month denervated muscle and to less extent in 7-month denervated muscle. The numbers of nuclei of abnormal morphology containing condensed and/or irregular patterns of chromatin distribution, as revealed by DNA staining and electron microscopy, exceed by 30-40 times the numbers of nuclei positive for the TUNEL reaction. In contrast to previous results [60, 93], a high discrepancy between frequency of morphological markers of apoptosis and DNA fragmentation seems a peculiar trait of myofiber death in rat denervated muscle.

Degeneration. Muscle *degeneration* (or *dystrophy*, as for genetic diseases) is a later effect of denervation: a significant portion of the muscle mass is substituted by different cells, mainly adipocytes (fat degeneration or lipodystrophy) and/or fibroblasts and collagen sheets around myofibers (endomysial fibrosis).

Generation and regeneration of myofibers

Mature mammalian skeletal muscle fibers maintain a relatively finite, fiber type-specific relationship between the size of the myofiber (e.g., myofiber length, cross-sectional area, or cytoplasmic volume) and the number of myonuclei present in a given myofiber [2, 29, 40]. The concept that there is a finite myonuclear domain size associated with myofibers predicts that hypertrophying myofibers must increase their myonuclear number to accommodate the enlargement process. However, shortly after birth, mammalian myofibers are permanently differentiated and thus cannot undergo mitotic division or directly increase their myonuclear number (i.e., by myonuclear division) [28]. Therefore, myofibers undergoing hypertrophy appear to require an external source of new nuclei to maintain or reestablish a relatively constant myonucleus-to-fiber size ratio [28, 29, 97]. There is a significant body of evidence to suggest that satellite cells are the source of new myonuclei in mature mammalian skeletal muscles. There is also some compelling evidence indicating that satellite cell proliferation is required to support the process of compensatory hypertrophy in mammalian skeletal muscle: when radiation is used to prevent satellite cell proliferation before initiation of skeletal muscle functional overloading the hypertrophy response is absent (2, 70, 79, 81].

Light and electron microscopy shows that the long term denervated muscle maintains a steady-state severe atrophy for the animal's life span, some morphological and molecular features indicating that events of aneural regeneration occur continuously [20, 22, 64]. In contrasts with older reports, permanently denervated muscle of rodents shows spontaneous regenerative myogenesis [22]. Formation of new muscle fibers is present as early as one month after surgery and reaches its maximum between 2 and 4 months following denervation. Then myogenesis gradually decreases with progressive post-denervation atrophy and degeneration.

The myogenic response is biphasic and includes two distinct processes. The first process resembles the formation of secondary and tertiary generations of myotubes during normal muscle development and dominates during the first two months of denervation. The activation of this type of myogenic response (*myofiber generation*, which increases the number of myofibers present in an anatomically defined muscle, *hyperplasia*) does not depend on cell death and degenerative processes *per se* [13].

A second type of myogenesis is a typical *regenerative* reaction that occurs mainly within the spaces surrounded by the basal lamina of dead muscle fibers [13, 22]. Myofibers of different sizes are susceptible to degeneration and death, which indicates that cell death in denervated muscle does not correlate with levels of muscle cell atrophy [13]. These regenerative processes

frequently result in development of abnormal muscle cells that branch or form small clusters surrounded by two layers of basal lamina (the old and the new one, which is secreted by the new myofibers) [13, 22, 64]. Spontaneous myofiber regeneration in long-term denervation has been quantified and shown to be non-compensatory and to result in reduction of satellite cell pools [10, 13, 30, 46, 52, 53, 78, 84, 102]. The turnover of myonuclei (but not necessarily of regenerating myofibers) studied by means of continuous infusion of 5-bromo-2-deoxyuridine (BRDU) is at most 1 to 2% per week in adult rats [78, 85]. Evidence of satellite cell depletion in denervated muscle rise some problems on long-term potential of regenerative myogenesis [30, 46]. On the other hand myotoxic-induced myogenesis speaks in favor of a long lasting potential (see below).

Interspecies differences rise additional doubts, since there are large differenced in post-denervation effects, even in the case of rate of atrophy [42, 51, 89]. In man denervation atrophy progresses at relatively slow rate in comparison to rat, but our observation that the myogenic reactions to denervation of the human muscle are very similar to those well described in rat, is heartening as to the potential application in patients' rehabilitation (see below).

Isogenes' switching (fiber typing)

After the fully differentiated pattern of fast and slow myosins has been established in normal adult skeletal muscles, acute denervation is of very little influence on the type of contractile proteins synthesized in early atrophying muscle fibers. Preferential atrophy of fast fibers followed by atrophy of slow fibers appears to be the typical feature of the early phases of denervation, producing only a small unbalance in fiber typing (reviewed in [69, 91, 99]). However during several months of permanent denervation there is an almost complete transformation of rat mixed muscles into almost pure fast muscles [17, 18, 21], the residual slow myosin being present with fast myosin in single myofibers [20, 91]. Analyses of denervated and aneurally regenerated muscles suggest that in long-term denervation of rat soleus the slow-to-fast transformation is mainly the consequence of repeated cycles of cell death and regeneration [52, 84].

Induced regenerative myogenesis in long-term denervation

Beside spontaneous events, regeneration of muscle fibers is studied after induction of muscle damage and regeneration by autografting or by myotoxins' treatment. Permanent denervation does not prevent induced muscle regeneration [64] and a long-term retention after denervation of this capability has been demonstrated: Bupivacaine induce in few days massive and synchronous myofiber regeneration of four-month denervated fast and slow rat muscles [19, 24]. Autografting of seven-month denervated rat muscles is followed by substitution of old fibers by new fibers five-day after surgery when the denervated muscles are treated with marcaine or notexin [10, 38, 46, 55].

The aneurally regenerated myofibers grow in two weeks up to one fourth of their normal adult fiber sizes [19, 23, 24, 64], then they undergo to denervation atrophy, which reach a steady-state at less than one tent of the normal fiber size.

When a series of injections of notexin was made in mouse muscles, leaving time for regeneration to occur between each injection, new muscle fibers were formed after up to four notexin treatments. This provides evidence that some of the myoblasts reenter an undifferentiated, stem cell-like state and are capable of myogenesis after further injuries to the muscle [34].

Short-term denervated muscles recover well after reinnervation, but after longer periods of denervation, variously estimated at 6 to 18 months in humans [4, 7], and 4 to 7 months in rats, denervated muscles do not become completely restored even when motor nerves regenerate into the muscles. The functional recovery of the long-term denervated muscles is significantly improved when the myofibers are induced to regenerate with a myotoxic anesthetic. Seven-month after denervation the rat EDL treated with marcaine not only regenerate, but opposite to the denervated degenerated (fibrotic) control is reinnervated by implantation of nerve. The finding that denervated muscles are capable of being restored to a significant level by inducing regeneration may be useful in the clinical treatment of denervated muscles [10].

Satellite cell proliferation and myofiber regeneration is enhanced in long-term denervated muscles subjected to electrical stimulation [5, 35, 36, 63, 72].

Electrical Stimulation of Denervated Muscles

Early after denervation

Many independent experiments have shown that when applied early after denervation electrical stimulation with different pattern of impulses modulates trophism and contractile characteristics to almost full conversion from fast-to-slow types or viceversa (for review see [39]).

Contractile phenotype of muscle fibres is under control of hormones, stretch and influences from the motoneurons. Motoneurons can affect muscle fibres by releasing neurotrophic substances and by evoking electrical activity in the muscle. For regulating contractile properties such as speed, strength and endurance it has been demonstrated that the signal to change is coded in the pattern of electrical activity [39, 82]. Thus, high amounts of activity lead to slow shortening velocity and myosin heavy chains, while low amounts of activity lead to a fast phenotype. Even slow myosin heavy chains could be expressed in denervated fast myofibers by slow-like continuous electrostimulation [18, 57, 98]. For regulation of twitch duration frequency also plays a role, and for preventing atrophy in denervated muscles high frequency seems to be beneficial, particularly in fast muscles. Recent evidence shows that trophism and

fiber type are regulated independently through different transduction signaling pathways [44, 67, 86]. The role of putative neurotrophic substances remains unclear, with the exception of release of neuronal agrin, which is relevant in modulation of neuromuscular junctions [9].

Though still criticized by several members of the medical community (due to their naiveness in approaches and inexperience in the proper methodology), electrical stimulation applied early after nerve injury maintains eutropy and function of denervated muscle [41, 62, 94].

Late after denervation

Few interesting publications concern restorative effects of electrical stimulation of long-term denervated muscles [1, 54, 83]. Intermittent electrical stimulation at 100 Hz (one train of 60 pulses every minute) either maintains or restores in two months the force output to nearly normal values when stimulation starts at two, four or even nine months after sciatectomy. Long-term stimulation causes similar increases in muscle fiber cross sectional area in fast and slow muscles, and changes are identical when innervated muscles are stimulated in the same way [54].

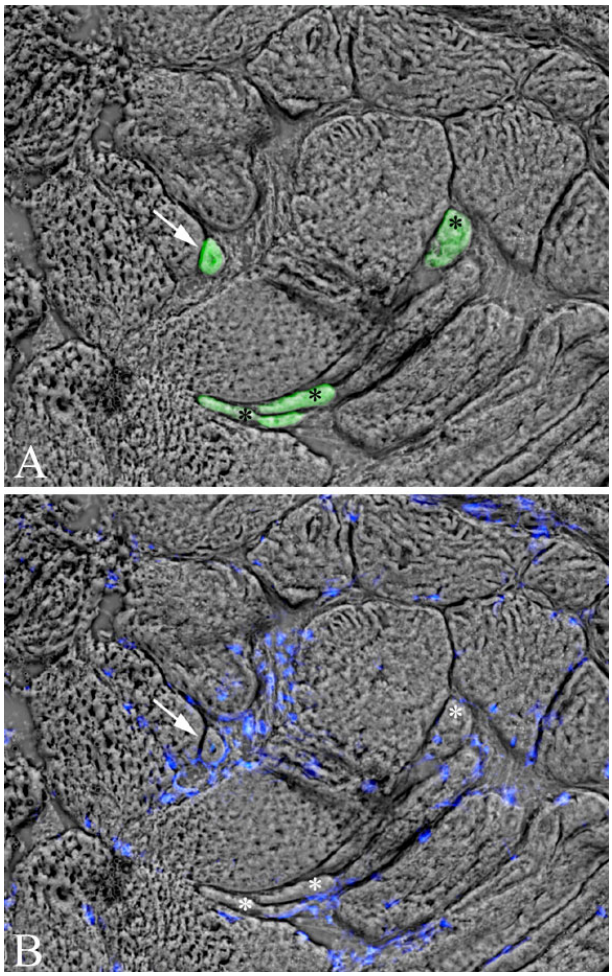

Figure 1. Myofiber regeneration in four-year denervated human muscle two-year after FES. About 3% of myofibers are stained by anti-embryonic myosin antibody (stars in Fig. 1 A). A few myofibers have central nuclei, a feature suggesting they are no more than 10-day old (arrow in Fig. 1, A and B).

Functional Electrical Stimulation (FES) of Long-Term Denervated Human Muscles

Over the last 30 years there has been a good deal of interest in the use of FES to restore movement of the limbs of patients immobilized by spinal-cord injury (upper motor neuron lesion, spastic paralysis) [68]. There is, however, another group of patients whose problems are more difficult to treat due to marked atrophy of denervated muscles and the associated loss of bone mass and skin atrophy causing severe secondary medical problems. In these patients injury also resulted in irreversible loss of the nerve supply to some or all of the affected limbs (flaccid paralysis due to lower motor neuron lesion). It is technically more difficult to treat these patients because direct stimulation of muscles requires more electrical energy than commercially available stimulation devices can deliver. The absence of functional nerve fibers makes it more difficult to recruit a sufficient population of myofibers to regain functional movements at an acceptable force level by surface electrodes. Despite these difficulties pilot studies on functional clinical application of FES on denervated muscles have been published. One has

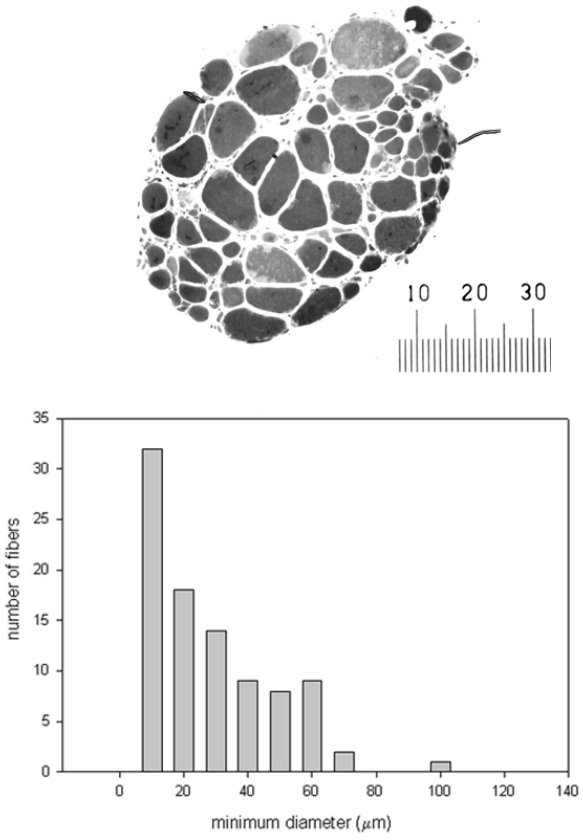

Figure 2. Bioptic muscle from four-year denervated human subject two-year after FES: Semithin section and frequency distribution spectrum of myofibers according to their minimum diameter.

demonstrated gait correction via direct FES of the denervated tibialis anterior muscle [94]. Two others, contrary to widely accepted opinion, have shown that electrical stimulation in such patients can restore muscle mass, force production and movement even after long-lasting complete denervation. Electrical stimulation reversed much of the deterioration undergone by the denervated tissues [48, 49, 99]. Muscle function in the lower extremities was restored sufficiently to support standing up, standing, and even a few steps [48, 49]. In addition to this functional restoration, improvements were observed in the condition of associated tissues (cartilage, tendons, bones, joints and skin). The associated stimulation equipment proofed to be effective for home-based training in a limited number of patients [43]. Recent analyses of biopsies from these patients confirm the presence of regenerating myofibers. In muscle biopsies of four-year denervated human subjects two-year after FES about 3% of myofibers are stained by anti-embryonic myosin antibody (stars in Fig. 1). A few myofibers have central nuclei, a feature suggesting they are no more than 10-day old (arrow in Fig. 1). Frequency distribution spectrum of myofibers according to their minimum diameter in semithin sections shows that about 50% are severely atrophic (minimum diameter smaller than 10 μm), but a large proportion of myofibers are eutrophic or hypertrophic, i.e. with a minimum diameter larger than 40 μm (Fig. 2).

Perspectives of Functional Electrical Stimulation (FES) of Human Denervated Regenerated Myofiber into Heart (Satellite Cell-Derived Cellular Cardiomyoplasty)

In skeletal-myoblast-based Cellular Cardiomyoplasty autologous myoblasts are the product of satellite cells grown in vitro from muscle biopsy of patients. Differentiation and fusion of proliferating myoblasts administered into myocardium produces myotubes, which may grow up to young myofibers containing significant and functional amount of contractile proteins, but finally they undergo post-denervation atrophy (aneural muscle regeneration), which could be in principle counteracted by electrical stimulation.

Evidence accumulated from animal studies has indicated that direct electrical stimulation of denervated muscles can to a large extent substitute for innervation and preserve or restore the normal properties of the muscles. Appropriate stimulation parameters are critical for successful interventions, and the best are obtained when the stimulation protocol resembles the firing pattern of the normal motoneurons (see for review [31]). In the patients electrical stimulation can restore muscle mass, force production and movement even after long-standing complete denervation. Electrical stimulation restores muscle function in the lower extremities sufficiently to support standing by reversing much of the deterioration undergone by the denervated tissues [48, 49].

Dynamic Cardiomyoplasty and related approaches demonstrated that sensing of heart cycle and synchronized stimulation of myofibers are safe procedures based on implanted electronics and batteries, which proved to last longer than five years [80].

Electronic engineers and bioengineers confronted with the clinical problems of activating and controlling motion of denervated muscles were able to solve major if not all the problems [32]. Myologists and neurobiologists are strenously working on reinnervation issues [16, 25, 33, 101]. No one tried the solution of artificial synapses, i.e. of a pool of miniaturized electrodes, which contact each of the surviving or regenerated myofibers in a denervated or in an engineered muscle. Taking advantage of the powerful angiogenesis of any developing tissue (including engineered myofibers in Cellular Cardiomyoplasty [47, 50, 61, 71, 73, 74, 90]) one may dream to use some of the new vascular branches to deliver sufficient current to new myofibers.

If artificial synapses could be designed and developed (to restrict activation to skeletal myoblast-derived myofibers, and avoid potentially arrhythmogenic excitability of the surrounding myocardiocytes) FES could preserve long-term healthful myofibers and add systolic assistance to benefits of satellite cell cardiac bio-assists.

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Note added in proofs

Recently, Hagege et al. [38] shown that, at autopsy 17.5 months after implantation of autologous skeletal myoblast in post-infarction scar, the derived myotube-like skeletal muscle fibers are viable, and longitudinally co-aligned with the adjacent myocardium. The severely atrophic myofibers expressed either fast (35%) or slow (32%), or co-expressed both slow and fast (33%) myosin isoforms.

References

[1] al-Amood WS, Lewis DM, Schmalbruch H: Effects of chronic electrical stimulation on contractile properties of long-term denervated rat skeletal muscle. *J Physiol (London)* 1991; 441: 243-256.

[2] Allen DL, Monke SR, Talmadge RJ, Roy RR, Edgerton VR: Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibers. *J Appl Physiol* 1995; 78: 1969-1976.

[3] Anderl H: Cross-face nerve grafting - up to 12 months of seventh nerve disruption, in Rubin LR (ed): *Reanimation of the Paralyzed Face*. St Louis, Mosby Publishers, 1977, pp 241-177.

[4] Andersen JL, Mohr T, Biering-Sorensen F, Galbo H, Kjaer M: Myosin heavy chain isoform transformation in single fibres from m. vastus lateralis in spinal cord injured individuals: effects of long-term functional electrical stimulation (FES). *Eur J Physiol* 1996; 431: 513-518.

[5] Apel ED, Lewis RM, Grady RM, Sanes JR: Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. *J Biol Chem* 2000; 275: 31986-31995.

[6] Bateman JE: *Trauma to Nerves in Limbs*. Philadelphia, Saunders Publishing, 1962.

[7] Batista RJV: Ventriculectomy for diameter heart surgery. *Basic Appl Myol* 1997; 7: 57-60.

[8] Bezakova G, Rabben I, Sefland I, Fumagalli G, Lomo T: Neural agrin controls acetylcholine receptor stability in skeletal muscle fibers. *Proc Natl Acad Sci USA* 2001; 14; 98 (17): 9924-9929.

[9] Billington L, Carlson BM: The recovery of long-term denervated rat muscles after Marcaine treatment and grafting. *Anat Rec* 1996; 144 (1-2): 147-155.

[10] Bolotin G, van der Veen FH, Lorusso R, Schreuder JJ, Wolf T, David JB, Uretzky G: Aortomyoplasty. *Basic Appl Myol* 1998; 8: 59-65.

[11] Borisov AB, Carlson BM: Cell death in denervated skeletal muscle is distinct from classical apoptosis. *Anat Rec* 2000; 258 (3): 305-318.

[12] Borisov AB, Dedkov EI, Carlson BM: Interrelations of myogenic response, progressive atrophy of muscle fibers, and cell death in denervated skeletal muscle. *Anat Rec* 2001; 264: 203-218.

[13] Burton EA, Davies KE: Muscular dystrophy: reason for optimism? *Cell* 2002; 108: 5-8.

[14] Capouya ER, Gerber RS, Drinkwater DC Jr, Pearl JM, Sack JB, Aharon AS, Barthel SW, Kaczer EM, Chang PA, Laks H: Girdling effect of nonstimulated cardiomyoplasty on left ventricular function. *Ann Thorac Surg* 1993; 56: 867-871.

[15] Carlson BM, Borisov AI, Dekov EI, Dow D, Kostrominova TY: The biology and restorative capacity of long-term denervated skeletal muscle. *Basic Appl Myol* 2002; 12 (6): 247-254.

[16] Carraro U, Catani C, Belluco S, Cantini M, Marchioro L: Slow-like electrostimulation switches on slow myosin in denervated fast muscle. *Exp Neurol* 1986; 94: 537-553.

[17] Carraro U, Catani C, Degani A, Rizzi C: Myosin expression in denervated fast- and slow-twitch muscles: Fiber modulation and substitution, in Pette D (ed): *The dynamic state of muscle fibers*. Berlin, New York, Walter de Gruyter, 1990, pp 247-262.

[18] Carraro U, Catani C: A sensitive SDS PAGE method separating heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem Biophys Res Commun* 1983; 116: 793-802.

[19] Carraro U, Dalla Libera L, Catani C, Danieli-Betto D: Chronic denervation of rat diaphragm: selective maintenance of adult fast myosin heavy chains. *Muscle Nerve* 1982; 5: 515-524.

[20] Carraro U, Dalla Libera L, Catani C: Myosin light and heavy chains in muscle regenerating in absence of the nerve: transient appearance of the embryonic light chains. *Exp Neurol* 1983; 79: 106-117.

[21] Carraro U, Morale D, Mussini I, Lucke S, Cantini M, Betto R, Catani C, Dalla Libera L, Danieli-Betto D, Noventa D: Chronic denervation of rat diaphragm: maintenance of fiber heterogeneity with associated increasing uniformity of myosin isoforms. *J Cell Biol* 1985; 100: 161-174.

[22] Carraro U, Rossini K, Zanin ME, Carraro U, Rossini K, Zanin ME: 2002. Muscle stem cells and recovery of function by electrical stimulation of permanent denervated, regenerated muscle. *Proceedings of the 7th Annual Conference of IFESS*. Ljubljana, Slovenia, June 25-29, 2002, pp 402-404. Conference Web Page at: http://robo.fe.uni.lj/ifess2002.

[23] Carraro U, Rossini K, Zanin ML, Rizzi C, Mayr W, Kern H: Induced myogenesis in long-term permanent denervation: perspective role in Functional Electrical Stimulation of denervated legs in humans. *Basic Appl Myol* 2002; 12: 53-64.

[24] Ceballos D, Valero-Cabre A, Valderrama E, Schuttler M, Stieglitz T, Navarro X: Morphologic and functional evaluation of peripheral nerve fibers regenerated through polyimide sieve electrodes over long-term implantation. *J Biomed Mater Res* 2002; 60 (4): 517-528.

[25] Chachques JC, Marino JP, Lajos P, Zedgi R, D'Attelis N, Fornes P, Fabiani JN, Carpentier A: Dynamic cardiomyoplasty: clinical follow-up at 12 years. *Eur J Cardiothorac Surg* 1997; 12: 560-567.

[26] Chachques JC, Shafy A, Cattadori B, Duarte F, Shen L, Meimoun P, Argyriadis P, Bruneval P, Carpentier A: Electrostimulation enhanced fatigue resistant myosin expression in cellular cardiomyoplasty. *Circulation* 2001; 104 (Suppl 2): 555-556.

[27] Chambers RL, McDermott JC: Molecular basis for skeletal muscle regeneration. *Can J Appl Physiol* 1996; 21: 155-184.

[28] Cheek DB, Holt AB, Hill DE, Talbert JL: Skeletal muscle mass and growth: the concept of the deoxyribonucleic acid unit. *Pediatr Res* 1971; 5: 312-328.

[29] DedkovAR, Kostrominova TY, Borisov AB, Carlson BM: Reparative myogenesis in long-term denervated skeletal muscles of adult rats results in a reduction of the satellite cell population. *Anat Rec* 2001; 263: 139-154.

[30] Eberstein A, Eberstein S: Electrical stimulation of denervated muscle: is it worthwhile? *Med Sci Sports Exerc* 1996; 28: 1463-1469.

[31] Elefteriades JA, Quin JA, Hogan JF, Holcomb WG, Letsou GV, Chlosta WF, Glenn WW: Long--term follow-up of pacing of the conditioned diaphragm in quadriplegia. *Pacing Clin Electrophysiol* 2002; 25 (6): 897-906.

[32] Fields DR, Stevens-Graham B: New insights into neuron-glia communications. *Science* 2002; 298: 556-562.

[33] Gross JG, Morgan JE: Muscle precursor cells injected into irradiated mdx mouse muscle persist after serial injury. *Muscle Nerve* 1999; 22 (2): 174-185.

[34] Grounds MD, White JD, Rosenthal N, Bogoyevitch MA: The role of stem cells in skeletal and cardiac muscle repair. *J Histochem Cytochem* 2002: 50: 589-610.

[35] Grounds MD: Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. *Annals NY Acad Sci* 1998; 854: 79-91.

[36] Gulati AK: Long-term retention of regenerative capacity after denervation of skeletal muscle, and dependency of late differentiation on innervation. *Anat Rec* 1988; 220: 429-434.

[37] Gundersen K: Determination of muscle contractile properties: the importance of the nerve. *Acta Physiol Scand* 1998; 162 (3): 333-341.

[38] Hagege AA, Carrion C, Menasche P, Vilquin J-T, Duboc D, Marolleau J-P, Desons M, Bruneval P: Viability and differentiation of autologous skeletal muoblast grafts in ischaemic cardiomyopathy. *Lancet* 2003; 361: 491-492.

[39] Hikida RS, Van Nostran S, Murray JD, Staron RS, Gordon SE, Kraemer WJ: Myonuclear loss in atrophied soleus muscle fibers. *Anat Rec* 1997; 247: 350-354.

[40] Hnik P, Skorpil V, Vyklicky L: Diagnosis and therapy of denervation muscle atrophy, in Gutmann E (ed): *The denervated muscle.* Prague, Publishing House of Czechoslovak Academy of Science, 1962, pp 433-466.

[41] Hnik P: Rate of denervation muscle atrophy, in Gutmann E (ed): *The denervated muscle.* Prague, Publishing House of Czechoslovak Academy of Science, 1962, pp 341-371.

[42] Hofer C, Mayr W, Stöhr H, Unger E, Kern H: A stimulator for functional activation of denervated muscles. *Artif Organs* 2002; 26 (3): 276-279.

[43] Hoover F, Kalhovde JM, Dahle MK, Skalhegg B, Tasken K, Lomo T: Electrical muscle activity pattern and transcriptional and posttranscriptional mechanisms regulate PKA subunit expression in rat skeletal muscle. *Mol Cell Neurosci* 2002; 19 (2): 125-137.

[44] Jain M, DerSimonian HD, Brenner DA, Ngoy S, Teller P, Edge A, Zawadzka A, Wetzel K, Sawyer DB, Colucci WS, Apstein CS, Liao RL: Cell therapy attenuates deleterious ventricular remodeling and improves cardiac performance after myocardial infarction. *Circulation* 2001; 103: 1920-1927.

[45] Jejurikar SS, Marcelo CL, Kuzon WM Jr: Skeletal muscle denervation increases satellite cell susceptibility to apoptosis. *Plast Reconstr Surg* 2002; 110 (1): 160-168.

[46] Kamihata H, Matsubara H, Nishiue T, Fujiyama S, Tsutsumi Y, Ozono R, Masaki H, Mori Y, Iba O, Tateishi E, Kosaki A, Shintani S, Murohara T, Imaizumi T, Iwasaka T: Implantation of bone marrow mononuclear cells into ischemic myocardium enhances collateral perfusion and regional function via side supply of angioblasts, angiogenic ligands and cytokines. *Circulation* 2001; 104: 1046-1052.

[47] Kern H, Hofer C, Mödlin M, Forstner C, Raschka-Höger D, Mayr W, Stöhr H: Denervated muscles in humans: limitations and problems of currently used functional electrical stimulation training protocols. *Artif Organs* 2002; 26 (3): 216-218.

[48] Kern H, Hofer C, Strohhofer M, Mayr W, Richter W, Stohr H: Standing up with denervated muscles in humans using functional electrical stimulation. *Artif Organs* 1999; 23 (5): 447-452.

[49] Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S: Neovascularization of ischemic myocardium by human bone-marrow-derived antioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nature Med* 2001; 7: 430-636.

[50] Lewis DM, al-Amood WS, Schmalbruch H: Effects of long-term phasic electrical stimulation on denervated soleus muscle: guinea-pig contrasted with rat. *J Muscle Res Cell Motil* 1997; 18 (5): 573-586.

[51] Lewis DM, Schmalbruch H: Contractile properties of aneurally regenerated compared with denervated muscles of rat. *J Muscle Res Cell Motil* 1994; 15 (3): 267-777.

[52] Lewis DM, Schmalbruch H: Effects of age on aneural regeneration of soleus muscle in rat. *J Physiol (London)* 1995; 488 (2): 483-492.

[53] Lomo T, Westgaard RH, Hennig R, Gundersen K: The response of denervated muscle to long-term electrical stimulation, in Carraro U, Angelini C, (eds): *Cell Biology and Clinical Management in Functional Electro Stimulation of Neurons and Muscles*. Padova, Italy, CLEUP Editore, 1985, pp 81-90.

[54] Lu DX, Huang SK, Carlson BM: Electron microscopic study of long-term denervated rat skeletal muscle. *Anat Rec* 1997; 248 (3): 355-356.

[55] Matsushita T, Oyamada M, Kurata H, Masuka S, Takahashi A, Emmoto T, Shiraishi I, Wada Y, Oka T, Takamatsu T: Formation of cell junctions between grafted and host cardiomyocytes at the border zone of rat myocardial infarction. *Circulation* 1999; 9: II262-268.

[56] Mayne CN, Mokrusch T, Jarvis JC, Gilroy SJ, Salmons S: Stimulation-induced expression of slow muscle myosin in a fast muscle of the rat. Evidence of an unrestricted adaptive capacity. *FEBS Lett* 1993; 327 (3): 297-300.

[57] McCarthy PM, Smith WA: Mechanical circulatory support: a long and winding road. *Science* 2002; 295 (5557): 998-999.

[58] McCarthy PM: Ventricular remodelling: Hype or hope? *Nat Med* 1996; 2: 859-860.

[59] Migheli A, Mongini T, Doriguzzi C, Chiado-Piat L, Piva R, Ugo I, Palmucci L: Muscle apoptosis in humans occurs in normal and denervated muscle, but not in myotonic dystrophy, dystrophinopathies or inflammatory disease. *Neurogenetics* 1997; 1 (2): 81-87.

[60] Miyagawa S, Sawa Y, Taketani S, Kawaguchi N, Nakamura T, Matsuura N, Matsuda H: Myocardial regeneration therapy for heart failure: hepatocyte growth factor enhances the effect of cellular cardiomyoplasty. *Circulation* 2002; 105 (21): 2556-2561.

[61] Mokrusch T, Engelhardt A, Eichhorn KF, Prischenk H, Sack G, Neundorfer B: Effects of long-impulse electrical stimulation on atrophy and fiber type composition of chronically denervated rabbit muscle. *J Neurol* 1990; 237: 29-34.

[62] Mussini I, Calliari I, Marchioro L, Vianello F, Gobbo V, Belluco S, Carraro U: Morphological changes of muscle fiber and neuromuscular junction following electrostimulation, in U Carraro (ed): *Sarcomeric and non-sarcomeric muscles: basic and applied research prospects for the 90's*. Padova, Unipress Padova, 1988, pp 391-402.

[63] Mussini I, Favaro G, Carraro U: Maturation, dystrophic changes and the continuous production of fibers in skeletal muscle regenerating in the absence of nerve. *J Neurophatol Exp Neurol* 1987; 46: 315-331.

[64] Oakley EL, Jarvis JC: Effects of a new cardiomyoplasty technique on cardiac function. *Cardiovasc Surg* 2001; 9: 50-57.

[65] Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P: Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; 410: 701-705.

[66] Pallafacchina G, Calabria E, Serrano AL, Kalhovde JM, Schiaffino S: A protein kinase B-dependent and rapamycin-sensitive pathway controls skeletal muscle growth but not fiber type specification. *Proc Natl Acad Sci USA* 2002; 99 (14): 9213-9218.

[67] Petrofsky JS, Stacy R, Laymon M: The relationship between exercise work intervals and duration of exercise on lower extremity training induced by electrical stimulation in humans with spinal cord injuries. *Eur J Appl Physiol* 2000; 82 (5-6): 504-509.

[68] Pette D: Historical Perspectives: plasticity of mammalian skeletal muscle. *J Appl Physiol* 2001; 90 (3): 1119-1124.

[69] Phelan JN, Gonyea WJ: Effect of radiation on satellite cell activity and protein expression in overloaded mammalian skeletal muscle. *Anat Rec* 1997; 247: 179-188.

[70] Pouzet B, Ghostine S, Vilquin J-T, Garcin I, Scorsin M, Hagege AA, Duboc D, Schwartz K, Menasche P: Is skeletal myoblast transplantation clinically relevant in the era of angiotensin-converting enzyme inhibitors? *Circulation* 2001; 104: I223-228.

[71] Putman CT, Dusterhoft S, Pette D: Satellite cell proliferation in low frequency-stimulated fast muscle of hypothyroid rat. *Am J Phisiol – Cell* 2000; 279 (3): C682-C690.

[72] Rajnoch C, Chachques JC, Berrebi A, Bruneval P, Benoit MO, Carpentier A: Cellular therapy reverses myocardial dysfunction. *J Thorac Cardiovasc Surg* 2001; 121: 871-878.

[73] Reinecke H, Poppa V, Murry CE: Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol* 2002; 34 (2): 241-249.

[74] Reinecke H, Zhang M, Bartosek T, Murry CE: Survival integration and differentiation of cardiomyocyte grafts. A study in normal and injured rat hearts. *Circulation* 1999; 100: 193-202.

[75] Reinlib L, Field L: Cell transplantation as future therapy for cardiovascular disease? *Circulation* 2000; 101: e182-187.

[76] Reinsch S, Gonczy P: Mechanisms of nuclear positioning. *J Cell Sci* 1998; 111: 2283-2295.

[77] Rodrigues AC, Schmalbruch H: Satellite cells and myonuclei in long-term denervated rat muscle *Anat Rec* 1995: 243 (4): 430-437.

[78] Rosenblatt JD, Yong D, Parry DJ: Satellite cell activity is required for hypertrophy of overloaded adult rat skeletal muscle. *Muscle Nerve* 1994; 17: 608-613.

[79] Salmons S: Permanent cardiac assistance from skeletal muscle: a prospect for the new millennium. *Artif Organs* 1999; 23: 380-385.

[80] Schiaffino S, Pierobon Bormioli S, Aloisi M: The fate of newly formed satellite cells during compensatory muscle hypertrophy. *Virchows Arch* 1976; B 21: 113-118.

[81] Schiaffino S, Reggiani C: Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol Rev* 1996; 76 (2): 371-423.

[82] Schmalbruch H, al-Amood WS, Lewis DM: Morphology of long-term denervated rat soleus muscle and the effect of chronic electrical stimulation. *J Physiol (London)* 1991; 441 (1): 233-241.

[83] Schmalbruch H, Lewis DM: A comparison of the morphology of denervated with aneurally regenerated soleus muscle of rat. *J Muscle Res Cell Motil* 1994; 15 (3): 256-266.

[84] Schmalbruch H, Lewis DM: Dynamics of nuclei of muscle fibers and connective tissue cells in normal and denervated rat muscles *Muscle Nerve* 2000; 23 (4): 617-626.

[85] Serrano AL, Murgia M, Pallafacchina G, Calabria E, Coniglio P, Lomo T, Schiaffino S: Calcineurin controls nerve activity-dependent specification of slow skeletal muscle fibers but not muscle growth. *Proc Natl Acad Sci USA* 2001; 98 (23): 13108-13113.

[86] Sola OM, Dillard DH, Ivey TD, Haneda K, Itoh T, Thomas R: Autotransplantation of skeletal muscle into myocardium. *Lab Invest* 1985; 71: 341-348.

[87] Starr DA, Han M: Role of ANC-1 in tethering nuclei to the actin cytoskeleton. *Science* 2002; 298: 406-409.

[88] Sutfin DC, Thomson JD, Hines HM: Kinetics of denervation atrophy in the skeletal muscles of the rat. *Am J Physiol* 1954; 178: 535-537.

[89] Suzuki K, Brand NJ, Allen S, Khan MA, Farrell AO, Murtuza B, Oakley RE, Yacoub MH: Overexpression of connexin 43 in skeletal myoblasts: relevance to cell transplantation to the heart. *J Thorac Cardiovasc Surg* 2001; 122: 759-766.

[90] Talmadge RJ, Roy RR, Bodine-Fowler SC, Pierotti DJ, Edgerton VR: Adaptations in myosin heavy chain profile in chronically unloaded muscles. *Basic Appl Biol* 1995; 5: 119-134.

[91] Taylor DA: Cellular cardiomyoplasty with autologous skeletal myoblasts for ischemic heart disease and heart failure. *Curr Control Trials Cardiovasc Med* 2001; 2 (5): 208-210.

[92] Tews DS, Goebel HH, Schneider I, Gunkel A, Stennert E, Neiss WF: DNA-fragmentation and expression of apoptosis-related proteins in experimentally denervated and reinnervated rat facial muscle. *Appl Neurobiol* 1997; 23 (2): 141-149.

[93] Valencic V, Vodovnik L, Stefancic M, Jelnikar T: Improved motor response due to chronic electrical stimulation of denervated tibialis anterior muscle in humans. *Muscle Nerve* 1986; 9: 612-617.

[94] Viguie CA, Lu DX, Huang SK, Rengen H, Carlson BM: Quantitative study of the effects of long-term denervation on the extensor digitorum longus muscle of the rat. *Anat Rec* 1997; 248 (3): 346-354.

[95] Wagers AJ, Sherwood RI, Christensen JL, Weissman IL: Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 2002; 297: 2256-2259.

[96] Weiss RA: Transgenic pigs and virus adaptation. *Nature* 1998; 391: 327-328.

[97] White TP, Esser KA: Satellite cell and growth factor involvement in skeletal muscle growth. *Med Sci Sports Exerc* 1989; 21: S158-163.

[98] Windisch A, Gundersen K, Szabolcs MJ, Gruber H, Lomo T: Fast to slow transformation of denervated and electrically stimulated rat muscle. *J Physiol (London)* 1998; 510: 623-632.

[99] Woodcock AH, Taylor PN, Ewins DJ: Long pulse biphasic electrical stimulation of denervated muscle. *Artif Organs* 1999; 23 (5): 457-459.

[100] Yoo K-J, Li R-K, Weisel RD, Donald AG, Mickle MD, Jia Z-Q, Kim E-J, Tomita S, Terrance M, Yau MD: Heart cell transplantation improves heart function in dilated cardiomyopathic hamsters. *Circulation* 2000; 102: III204-209.

[101] Yoshii S, Oka M, Shima M, Taniguchi A, Akagi M: 30 mm regeneration of rat sciatic nerve along collagen filaments. *Brain Res* 2002; 949 (1-2): 202-208.

[102] Yoshimura K, Harii K: A regenerative change during muscle adaptation to denervation in rats. *J Surg Res* 1999; 81 (2): 139-146.