

Fundamentals of Transformed Skeletal Muscle Used for Cardiac Assistance. An Overview

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ABSTRACT

Striated skeletal muscles transformed into fatigue-resistant units by chronic electrostimulation are commonly used in cardiomyoplasty and other techniques of circulatory assistance. In view of its importance, the fundamentals of muscle transformation are briefly reviewed to emphasize the potential and limitations of transformed muscle. An improved understanding of this remarkable plasticity phenomenon could contribute to optimize current experimental and clinical applications.

Key words: Striated skeletal muscle. Electrostimulation. Cardiomyoplasty.

Skeletal muscles consist in variable proportions of type I and type II fibers, thus accounting for the classical distinction between red and pale muscles. Myoglobin and cytochrome confer a red appearance to muscles involved in sustained tonic contractions, such as the soleus. Such muscles are characterized by a predominance of type I fibers that are mainly oxidative, resistant to fatigue, and slow twitching. Fast twitching, glycolytic, fatigue-prone type II (IIa, IIb, IIc) fibers are encountered in pale muscles. The latissimus dorsi, in both man and goat, is constituted by a mixed population of type I ($\pm 30\%$) and type II ($\pm 70\%$) fibers (Fig. 1a).

The idea of using striated muscle for cardiovascular support has evolved in three main stages. Thus, initially, unstimulated muscle flaps were applied onto the heart to reinforce the cardiac wall, or in the hope of increasing myocardial blood supply (1-3).

In a second phase, acutely electrostimulated muscles were used in the experimental setting, with interesting results that were nevertheless soon hindered by muscle fatigue, incomplete muscle contraction during stimulus, and subsequent muscle fibrosis and degeneration (4-9). This was mainly due to the use of unsuitable stimulating devices and stimulation protocols

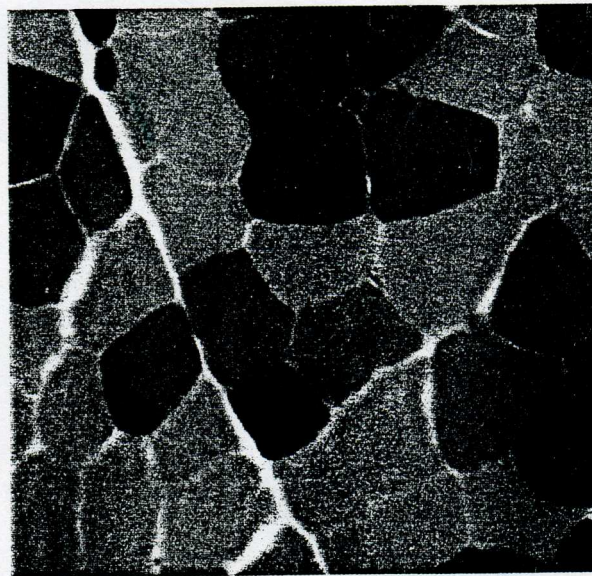


Fig. 1a. Histochemical study of ATPase activity (pH 4.35, native goat latissimus dorsi (x200). Dark stained fibers are type I fatigue-resistant fibers, while clear ones are type II

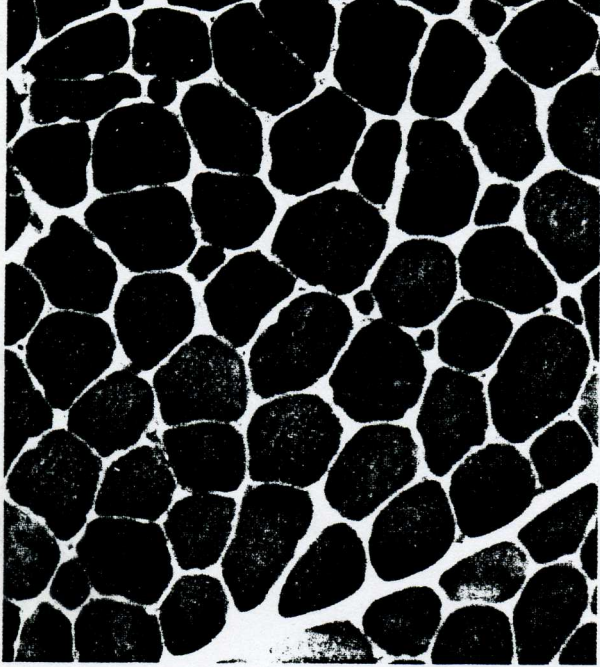


Fig. 1b. Histochemical study of ATPase activity (pH 4.35) in the same muscle as in Fig. 1a, after 8 weeks of stimulation ($\times 120$). A homogeneous population of type I fibers is observed.

Finally, it was not until recently that two fundamental advances led to the definitive recognition of the relevance of the concept. First, the work of Buller et al. (10) was revisited. Using an elegant model of cross reinnervation between fast- and slow-twitch muscles, these authors pointed out the phenotypic changes resulting from modifications in patterns of neural influx. This contribution shed light on the potential plasticity of striated skeletal muscles and boosted fundamental research in the field. Salmons et al. (11,12) and Pette et al. (13), studying chronically stimulated muscles demonstrated that fatigue resistance - which results from morphological, biochemical, and physiological modifications - takes place as an adaptive response to a change in functional demand. The second fundamental advance was the development by the group of Broussais Hospital and the Bakken Research Center (14) of a completely implantable and teleprogrammable burst stimulator, synchronized to cardiac activity, that contributes to match muscular contraction to systole - thus allowing effective assistance. Simultaneously, platinum-iridium alloy electrodes of long durability were developed. These juxtaneural electrodes are implanted into the skeletal muscle, with a large surface area, thereby facilitating modulable homogenous muscle contraction via nerve fiber depolarization (15).

Striated skeletal muscle flaps transformed into fatigue resistant units were then applied experimentally and clinically around the heart in cardiomyoplasty (16,17), or were used to power cardiac assist devices such as the

systems (17,20). A study by Acker et al. (21) consistently showed that skeletal muscle has the potential to work efficiently in a cardiac assist role. The first clinical application was based on the cardiomyoplasty procedure, introduced in 1985 by Carpentier and Chachques (16). Since then, about 300 procedures have been performed worldwide, with encouraging early results. These last authors also introduced experimentally and clinically (in 1988 and 1992, respectively) a new technique of diastolic assistance (aortomyoplasty).

The purpose of this paper is to review - in the light of the recent literature and our own experimental data obtained in the goat model - the fundamentals of plasticity accounting for fast-to-slow transformation, along with certain implications regarding transformed skeletal muscle involvement in circulatory assistance (cardiomyoplasty and aortomyoplasty). We emphasize the intrinsic possibilities and limitations due to constraints imposed by the cardiovascular system, and the new characteristics of the fatigue resistant muscles.

Metabolic adaptations of chronically stimulated muscles

Increased workload resulting from low-frequency chronic stimulation induces profound changes in oxidative and glycolytic anaerobic metabolism. Despite species specificities, general trends may be recognized.

Enzymatic markers of anaerobic metabolism (glycogen phosphorylase, phosphoglucosmutase, fructose 1-6 diphosphatase, and lactate dehydrogenase (LDH)) decrease within the first weeks of stimulation, reaching values similar to those of slow-twitch muscles such as the soleus (22,23). The preponderant muscular (M) isoforms of LDH shift to the H form (encountered in the heart), due to the synthesis of new H subunits (24)(Fig. 2). Creatine kinase and adenylate kinase enzymes involved in phosphoryl group transfer, and ATP regeneration in the cytosol, undergo significant decreases (13).

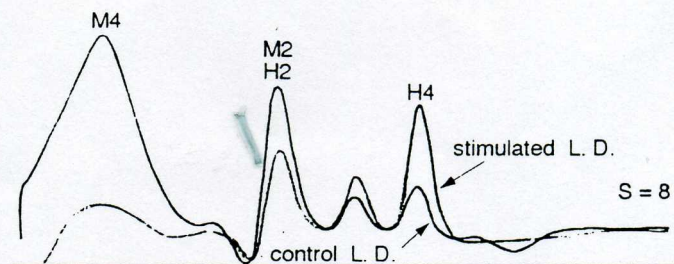


Fig. 2. Gel electrophoresis (SDS PAGE) of LDH in native (dark lines) and stimulated latissimus dorsi (dim lines). Native latissimus is characterized by a preponderance of the M4 isoform, while stimulated muscle mostly exhibits the H4 isoforms (8 weeks of stimulation).

While anaerobic glycolytic metabolism appears to regress, preponderance of the oxidative pathway is observed. This leads to an increase in both O_2 consumption and mitochondrial as well as capillary

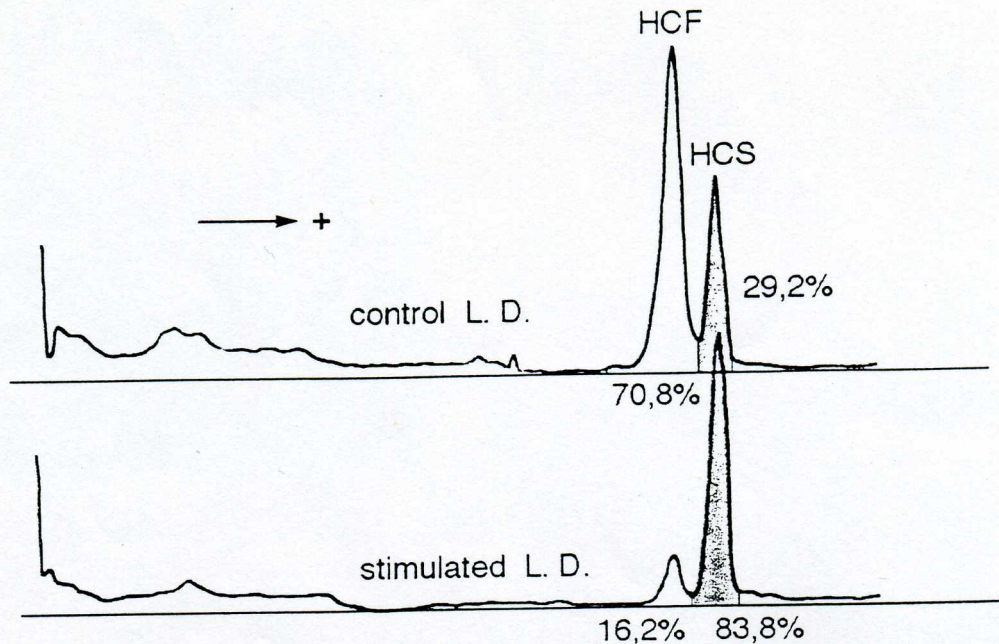


Fig. 3. Gel electrophoresis (SDS PAGE) of myosin heavy chains in controlled (top) and stimulated latissimus dorsi (bottom). Evidence is observed of a shift from myosin heavy chain fast (HCF) in native latissimus dorsi (LD) to a predominance of myosin heavy chain slow (HCS) after 8 weeks of stimulation.

density (12,25). The enzymes of the Krebs cycle (succinate dehydrogenase, citrate synthase and malate dehydrogenase) promptly increase following stimulation and reach, after 10 weeks, the values encountered in slow-twitch muscles (13,22). Cytochrome oxidase - the terminal enzyme of the respiratory chain - undergoes a three-fold increase after 5 weeks of stimulation (26). Concomitantly, enzymes involved in fatty acid breakdown (beta-oxidation) and oxidation of ketone bodies are enhanced (22). This is illustrated by the increase in 3-hydroxy Acyl CoA dehydrogenase, a key enzyme of beta-oxidation, which exhibits a time pattern similar to that of succinate dehydrogenase. The level of 3-oxoacid CoA transferase - the first enzyme of ketoacid metabolism - rises faster than the enzymes of the Krebs cycle. The activity of carnitine acyltransferase, involved in the transfer of fatty acids across the mitochondrial membrane, is also increased.

The activities of mitochondrial oxidases, NADH cytochrome c oxidoreductase (NADH cyt c OR), succinate cytochrome c oxidoreductase (succ cyt c OR), rise within the first days of stimulation to a peak three- to four-fold the basal values - with a secondary decrease to stable values superior to those seen initially (27,28). Since the chemosmotic complexes I and III represent the converging point of all reducing equivalents produced by cellular dehydrogenases, activities of these complexes are interesting indicators of the last steps of the oxidative metabolism.

Such modifications occur as a consequence of different types of stimulation protocol and confer, at least in part, fatigue resistance. Many authors believe that the increase in oxidative capacity illustrated by the increase in the major enzymes of the tricarboxylic acid cycle, as well as enhanced activity of beta-oxidation and ketone acid

crucial role (25). Unfortunately, the overall regulation of the relative importance of aerobic and anaerobic metabolism is incompletely understood. Indeed, we lack knowledge as to the exact role of mitochondrial translocases in the regulation of the metabolic flux between glycolysis and mitochondrial oxidative metabolism which they connect. Pyruvic translocase and malate-aspartate shuttle (composed of two carriers and four enzymes) establish this connection. They promote the transfer into the mitochondria of pyruvate (terminal oxidized product of glycolysis which enters the Krebs cycle after oxidative decarboxylation) and the reducing equivalent of NADH. Clearly, these enzymatic systems are in competition with cytosolic LDH for pyruvate and NADH, respectively. An increase in their activity lowers the sarcoplasmic production of lactate. Although the activity of the four soluble enzymes of the malate-aspartate shuttle increases following exercise (29), it is not known whether these enzymes, or the carriers, exercise a high level of control over the metabolic flux passing through them. The study of these elements of the malate-aspartate shuttle is currently the center of our attention.

Adaptations of the contractile apparatus

The interaction of actin and myosin in the sarcomere, fuelled by ATP, forms the basis of muscle contraction. During transformation from fast to slow-twitch fatigue-resistant muscles, consistent modifications in calcium metabolism have been recorded. This divalent cation plays a crucial role in the coupling of the electric stimulus (depolarization of the sarcolemma) to mechanical events. After two weeks of stimulation, the volume occupied by the sarcoplasmic reticulum significantly decreases, along with sarcoplasmic associated Ca^{2+} ATPase activity (30).

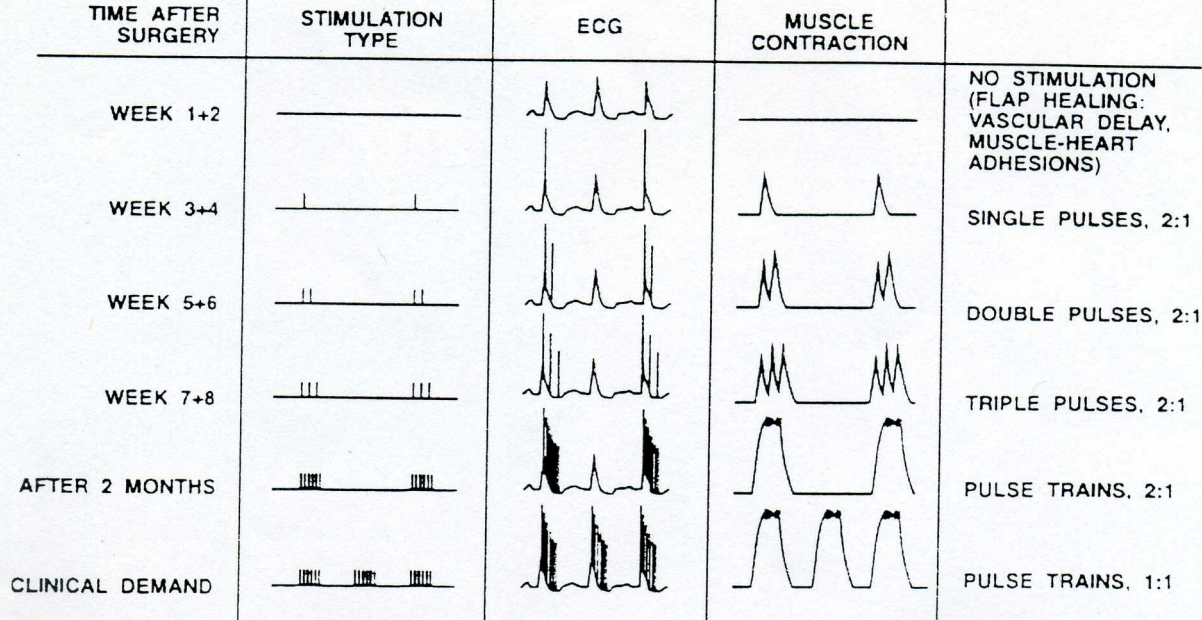


Fig. 4. Illustration of the progressive stimulation protocol developed at Broussais' Hospital. Synoptic view of the stimulating impulses, electrocardiographic recordings and mechanical muscular events.

(parvalbumin) and of calsequestrin in the sarcoplasmic reticulum was further demonstrated (31). These changes account for a reduced speed of calcium liberation and uptake, explaining prolongation of muscle relaxation and slower contraction.

Myosin is a major factor in determining the contractile properties of striated skeletal muscle, partly because the velocity of contraction is correlated to the ATPase activity of myosin (32-34). This protein exhibits several isoforms of its heavy and light chains. During adaptation to an increased workload, the fast myosin encountered in fast-twitch type II fibers is replaced by the slow isoform characteristic of type I fibers (24,35)(Fig. 3). These modifications can easily be quantified by electrophoresis (SDSPAGE) or by histoenzymological techniques. The conversion process can be extremely efficient. Starting with 30% type I fibers and 70% type II fibers, a complete transformation into slow isoforms is observed within 6 to 8 weeks (Fig. 1b).

Basic mechanics of electrostimulated skeletal muscle

An isolated skeletal muscle may be stimulated via its attached nerves or more directly by application of an electric impulse. A major feature of twitch fibers is that they are fully activated above a certain threshold stimulus strength, responding in an all-or-nothing mode. At the level of the whole muscle, however, the amplitude of contraction and the force delivered are regulated by the variations in spatial (some or all the fibers are activated) and temporal recruitment (fibers firing at their maximum rate). A single shock applied on the muscle provokes a twitch contraction. Increased frequency of shocks leads to fusion of the contractions that ultimately attain a maximum tension when fused tetanus is reached (i.e.,

maximal spatial and temporal recruitment). This fundamental feature of striated muscle has been used advantageously in the concept of cardiac assistance. While single impulses entrain a moderate muscle contraction, diffuse and vigorous muscle contraction is obtained by multiple spaced impulses (train of impulses). The duration of contraction is physiologically adapted to ventricular systole. A train of impulses of 30 Hz generates action potentials of similar frequency to that induced by physiologic nerve stimulation of cell depolarization at the motor end-plate of slow-twitch fibers (type I), and produces fused skeletal muscle contraction. The cycle for chronic electromyostimulation, with 25% stimulation time (duration of stimulation: 185 msec.) and 75% recovery time (555 msec.) allows the generation and maintenance of repetitive muscle contraction without significant fatigue or muscle fibers degeneration (Fig. 4).

This constitutes the basis of cardiomyostimulation, in which an adequate stimulus activates the muscle following the sensing of intrinsic cardiac electrical activity. Muscles stimulated in this mode have been found to provide efficient assistance in experimental and clinical applications.

Relaxed muscles are readily extensible. In this condition, the actin and myosin filaments do not appear to interact, and the elasticity results from elastic components of the muscle (connective tissue, cell membrane, etc.). On stimulation, striated muscle, like myocardium, obeys the Starling law which states that the external constraint (i.e., the load) determines the force of contraction. Up to a limit, after which active tension (T) decreases, an increase in the load on the relaxed muscle results in an increase in the force of contraction (Fig. 5). If the muscle is held at a fixed length, it will develop tension. Such a process is

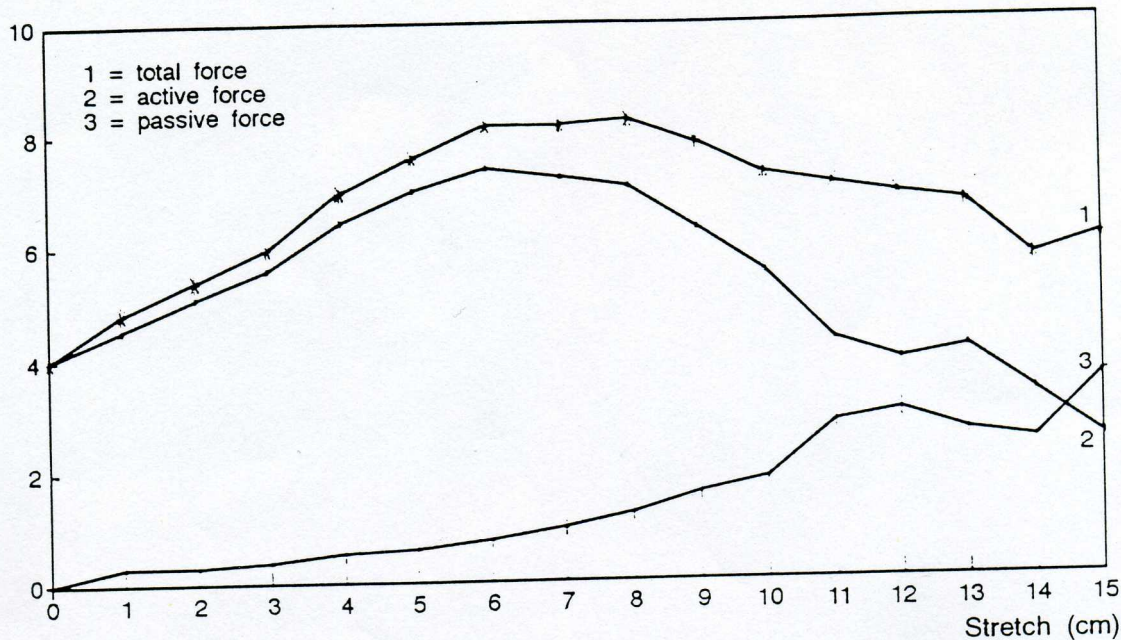


Fig. 5. Plot of total force (Curve 1), active force obtained by subtracting Curve 3 from Curve 1 (Curve 2), and passive force (Curve 3) of goat latissimus dorsi muscle (LD) tested in situ (after mobilization preserving the thoracodorsal bundle) in isometric strain gauge testing. The force-length relationship is depicted. As stretch (preload) progresses on the abscissas axis from 0 (corresponding to equilibrium length), the force delivered increases to a maximum point (stretch value 6-8). From there onwards, the total force decreases due to the drop in active force, while the passive force curve illustrates the behaviour of the muscular elastic component.

termed isometric contraction, even though the muscle does not actually shorten and does not accomplish external work. If the load is less than the isometric tension, the muscle will shorten. The steady velocity (V) of such an isometric contraction reaches a maximum at zero external load and declines to zero when the load matches the isometric tension. This force (tension)-velocity relation is fundamental in muscle mechanics. The power output ($T \times V$) reaches a maximum when T and V are about one third of their maximum values (27). Basically, skeletal muscle can perform more work per unit than cardiac muscle, both in cyclical and in single contraction, and can generate a force per unit cross sectional area that is between two- and five-fold higher (36). Therefore, it can theoretically sustain incessant pumping work or regular contraction, provided that the profound changes leading to fatigue resistance have occurred and loading conditions (which also depend on muscle configuration) are optimal.

It must be noted that the transformation process goes far beyond a mere increase in endurance. Major alterations have been found in both the power that the muscle is capable of delivering and in the working conditions under which it can perform to greatest advantage, thus delivering maximal power. Studies by Salmons et al. (36), using the rabbit tibialis anterior (10Hz - 11 weeks), revealed considerable modifications in the force velocity curves, with a marked reduction (8-fold) in the power output and a shortening of the velocity at which maximum power is attained. Nevertheless, even under these conditions it was found that muscle still has sufficient theoretical working capacity to achieve satisfactory cardiac assistance, provided the loading conditions enable the muscle to operate near

speed of contraction and relaxation are important changes to be taken into account, since they might prevent the muscle from reaching its full working capacity during tachycardia, and may impede diastolic cardiac filling when wrapped in cardiomyoplasty or applied as a skeletal muscle ventricle. However, current clinical and experimental results using burst stimulation (30Hz, 18 msec.) showed no evidence of constriction or diastolic restriction after procedures such as cardiomyoplasty.

Comment

The basic knowledge acquired during the past 20 year in chronically stimulated muscles make us confident of the value of striated skeletal muscle in cardiocirculator assistance. The current understanding of the biochemical and bioenergetic processes conducting to fatigue resistance, along with advances in muscle electric physiology and improvements in muscle configuration should lead to exploitation of the full potential of transformed skeletal muscle. Since the aim of complete transformation is probably not optimal, for it somewhat reduces the working potential of the muscle involved, it is hoped that improved knowledge of the factors participating in the dynamic equilibrium between workload, energy demand and generating capacity, and the efficacy of the contractile system, will allow the development of stimulation protocols that preserve optimal biomechanical characteristics without suppressing fatigue resistance. This opens a "stimulating" field for optimizing current clinical and experimental application of "transformed" skeletal muscle.

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