Muscle Injuries

Biology and Treatment

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Muscle injuries are one of the most common traumas occurring in sports. Despite their clinical importance, few clinical studies exist on the treatment of these traumas. Thus, the current treatment principles of muscle injuries have either been derived from experimental studies or been tested only empirically. Although nonoperative treatment results in good functional outcomes in the majority of athletes with muscle injuries, the consequences of failed treatment can be very dramatic, possibly postponing an athlete's return to sports for weeks or even months. Moreover, the recognition of some basic principles of skeletal muscle regeneration and healing processes can considerably help in both avoiding the imminent dangers and accelerating the return to competition. Accordingly, in this review, the authors have summarized the prevailing understanding on the biology of muscle regeneration. Furthermore, they have reviewed the existing data on the different treatment modalities (such as medication, therapeutic ultrasound, physical therapy) thought to influence the healing of injured skeletal muscle. In the end, they extend these findings to clinical practice in an attempt to propose an evidence-based approach for the diagnosis and optimal treatment of skeletal muscle injuries.

Keywords: skeletal muscle; injury; regeneration; satellite cell; scar formation; immobilization

NORMAL STRUCTURE OF MYOFIBER AND THE SURROUNDING CONNECTIVE TISSUE

Skeletal muscle can simplistically be considered to be composed of 2 main components, the myofibers and the connective tissue. The myofibers with their innervating nerves are responsible for the contractile function of the muscle, whereas the connective tissue provides the framework that binds the individual muscle cells together during muscle contraction and embraces the capillaries and nerves within the muscle structure. Skeletal muscle myofibers are ribbon-like cells with the length varying in humans from a few millimeters (m. stapedius) to up to 50 cm (m. sartorius) and the diameter ranging from 15 to 20 mm (extrinsic eye muscles) to more than 100 mm in trained power athletes. The connective tissue network, in turn, creates a supportive skeleton for the myofibers, summing up the contraction of individual fibers into a joint effort and, thus, converting the contraction of the individual myofibers into efficient locomotion. Each myofiber is attached at both ends to the connective tissue of a tendon or the tendon-like fascia at the so-called myotendinous junctions (MTJs).[155] The individual myofibers are bound together by a connective tissue structure composed of 3 levels of sheaths called the endomysium, perimysium, and epimysium.[146] The basic component of this structure is the endomysium (also called the basement membrane), a delicate sheath that surrounds each myofiber. The next level is the perimysium, which combines anywhere from some tens to a couple hundred individual myofibers together into larger structures called fascicles. Finally, the epimysium, a considerably stronger and thicker sheath, surrounds the entire muscle belly and is made up of fascicles.

The attachment of myofibers to the tendon/fascia has to possess the strength to withstand considerable forces up to 1000 kg during maximal workload.[153,155] To have such high tensile strength, each myofiber contains specific chains of molecules called the integrins and dystrophin-glycoprotein complex.[104,108] These protein complexes connect the contractile myofilament apparatus to the extracellular matrix (ECM) through the sarcolemma.[20,29,31,41,50,83,144] Integrins

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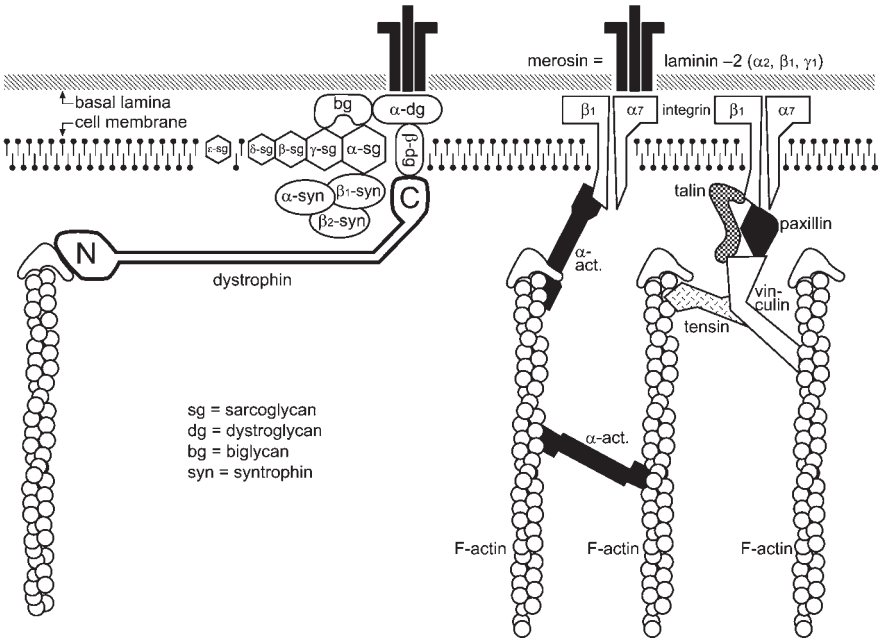


Figure 1. A schematic presentation of myofiber–extracellular matrix (ECM) adhesion. Each myofiber contains specific chains of molecules called the integrins and dystrophin, which connect the contractile myofilament apparatus to the ECM through the sarcolemma. The majority of integrins are located in the myotendinous junctions. The sarcomeric actin binds via several subsarcolemmally located molecules to the β1 subunit of the muscle-specific transmembrane integrin α7β1, which binds to the ECM proteins. The molecules of the dystrophin-associated complex are relatively evenly distributed along the entire sarcolemma, although they are particularly abundant in the myotendinous junctions and the neuromuscular junctions. The contractile protein actin binds to dystrophin, which is then associated with 3 protein complexes: the dystroglycans, the sarcoglycans, and the syntrophins.

are a family of adhesion receptors located on the cell membrane and have been shown to play a fundamental role in many biological processes related to tissue survival, growth, regeneration, and communication, such as the cell-cell signaling, the cell-ECM interactions, and the transduction of the signals in and out of the cell.[50,104,131]

In a healthy myofiber, the majority of integrins are located in the MTJs[9,83,84,104] in a specific structure termed the *integrin-associated complex* (Figure 1). In this complex, the terminal sarcomeric actin binds via several subsarcolemmally located molecules to the β1 subunit of the muscle-specific transmembrane integrin α7β1,[83,104,119,140,168] which then connects the intracellular contractile apparatus to the surrounding ECM by binding to the ECM proteins[23] (Figure 1). In contrast to the "accumulation" of the integrins at the ends of the myofiber, the molecules of the dystrophin-glycoprotein complex (Figure 1) are relatively evenly distributed along the entire sarcolemma, although they are more abundant in the MTJs and the neuromuscular junctions.[20,32,58,83,108,144] The contractile protein actin binds to dystrophin, which is then associated with 3 protein complexes, the dystroglycans, the sarcoglycans, and the syntrophins,[32,58,108] of which α-dystroglycan binds to the ECM proteins.[108]

MECHANISM OF SKELETAL MUSCLE INJURY

Muscle injuries are one of the most common injuries occurring in sports, with an incidence varying from 10% to 55% of all the sustained injuries.[10,48,73] Muscle injuries can be caused by contusion, strain, or laceration.[48,73] Muscle lacerations are the most uncommon of the muscle injuries occurring in sports, as more than 90% of all sports-related injuries are either contusions or strains.[73] A muscle contusion occurs when a muscle is subject to a sudden, heavy compressive force, such as a direct blow to the muscle. This kind of muscle trauma typically takes place in contact sports, whereas sprinting and jumping are the most common activities associated with muscle strains.[37,48] In strains, an excessive tensile force subjected onto the muscle leads to the overstraining of the myofibers and consequently to a rupture near the MTJ. Muscle strains typically concern the superficial muscles working across 2 joints, such as the rectus femoris, semitendinosus, and gastrocnemius muscles.[37,48,88,92]

THE PATHOBIOLOGY OF THE MUSCLE INJURY

What distinguishes a healing skeletal muscle from a healing injury in bone is that the skeletal muscle heals by a repair process, whereas the bone heals by a regenerative process. When most of the tissues in the body are injured, they will heal with a scar, which is a different tissue than was there before, whereas when a bone is broken, the healing tissue is identical to the tissue that existed there before. The healing of an injured skeletal muscle follows a fairly constant pattern irrespective of the underlying cause (contusion, strain, or laceration).[62,88] Three phases have been identified in this process[88]:

1. destruction phase, characterized by the rupture and ensuing necrosis of the myofibers, the formation of a hematoma between the ruptured muscle stumps, and the inflammatory cell reaction;
2. repair phase, consisting of the phagocytosis of the necrotized tissue, the regeneration of the myofibers, and the concomitant production of a connective tissue scar, as well as the capillary ingrowth into the injured area; and

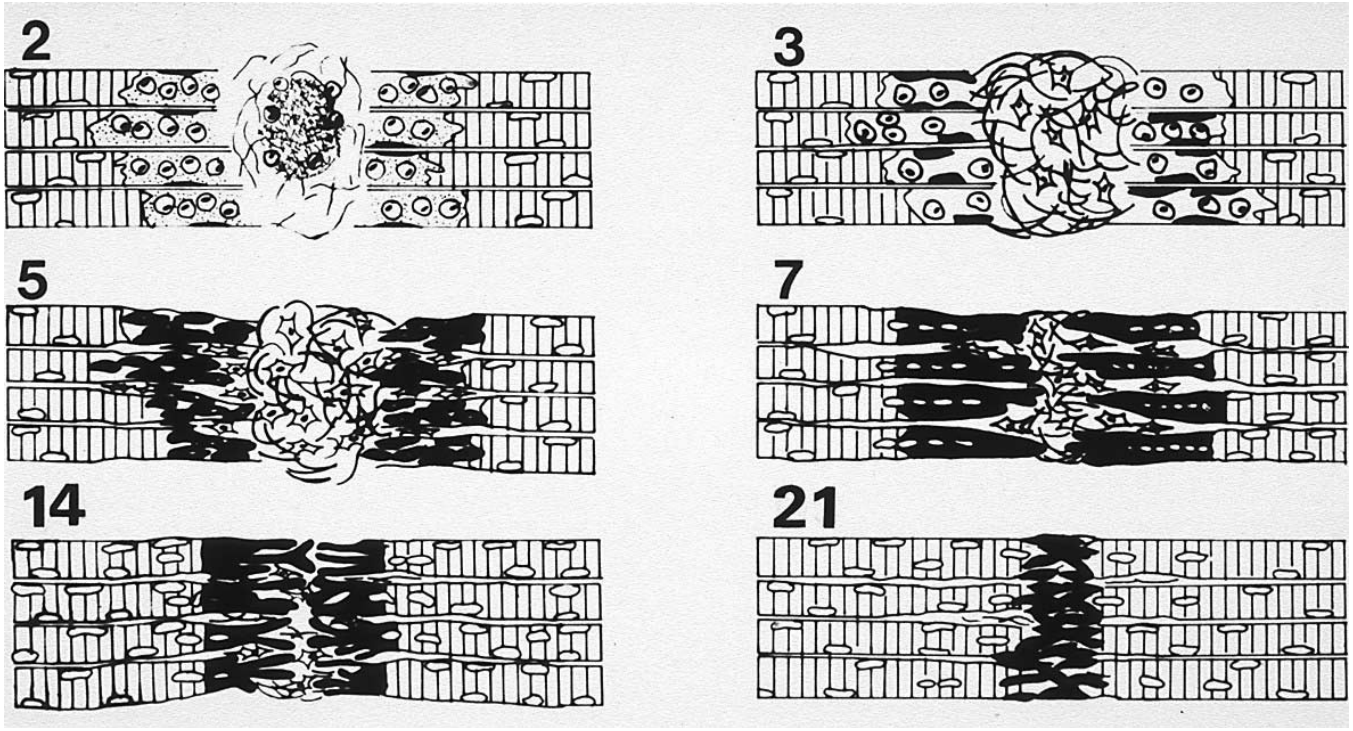


Figure 2. A schematic illustration of the healing skeletal muscle. Day 2: the necrotized parts of the transected myofibers are being removed by macrophages while, concomitantly, the formation of the connective tissue scar by fibroblasts has begun in the central zone (CZ). Day 3: satellite cells have become activated within the basal lamina cylinders in the regeneration zone (RZ). Day 5: myoblasts have fused into myotubes in the RZ, and the connective tissue in the CZ has become denser. Day 7: the regenerating muscle cells extend out of the old basal lamina cylinders into the CZ and begin to pierce through the scar. Day 14: the scar of the CZ has further condensed and reduced in size, and the regenerating myofibers close the CZ gap. Day 21: the interlacing myofibers are virtually fused with little intervening connective tissue (scar) in between.

3. remodeling phase, a period during which the maturation of the regenerated myofibers, the contraction and reorganization of the scar tissue, and the recovery of the functional capacity of the muscle occur.

The latter 2 phases—repair and remodeling—are usually closely associated or overlapping[88] (Figure 2).

DESTRUCTION PHASE

Rupture of Muscle

When a contusion type of external force causes an injury to a skeletal muscle, the rupture occurs at or adjacent to the impact site, whereas in muscle strains, the injury of an otherwise healthy muscle is usually located at either of its ends close to the MTJ.[15,48,49,96] In a contracted muscle, the contusion-induced injury is more superficial than in a relaxed muscle, in which the rupture is usually adjacent to the bone as the pressure of the impact is transmitted through the muscle layers until the muscle is compressed against the bone surface.[36,48,49]

Necrosis of Myofibers

When the muscle is injured, an excessive mechanical force usually extends across the entire cross section of the individual myofibers (Figures 2 and 3), subsequently tearing the sarcoplasm of the ruptured muscle stumps and then leaving it wide open. As the myofibers are very long, string-like cells, there is an imminent threat that the necrosis initiated at the site of injury extends along their entire lengths. However, there is a specific structure called the contraction band, a condensation of cytoskeletal material, that acts as a system of "fire doors."[62] Within hours after the injury, the propagation of necrosis is halted to a local process, as the contraction band seals off the defect in the plasma membrane and forms a protective barrier in which the torn plasma membrane can be repaired[62] (Figure 4). Recent studies have demonstrated that lysosomal vesicles that are inserted at the site of the disrupted plasma membrane as a temporary membrane play a pivotal role in the completion of the resealing of the plasma membrane.[105,111]

Inflammation

Along with an injury to the myofibers, the blood vessels of the muscle tissue are naturally torn, and thus, the blood-

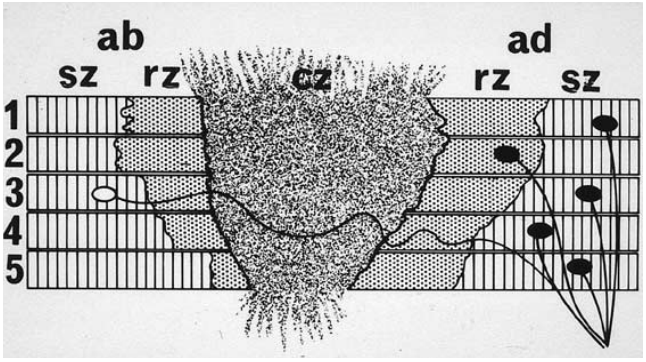


Figure 3. A schematic presentation of a shearing injury of skeletal muscle. The ruptured myofibers contract, and the gap between the stumps (central zone [CZ]) becomes initially filled by a hematoma. Myofibers are necrotized within their basal lamina over a distance of about 1 to 2 mm, within which segment a complete regeneration usually takes place with time (regeneration zone [RZ]), whereas only reactive changes are observed in the parts of the muscle surviving the trauma (survival zone [SZ]). Each myofiber is innervated at a single point of neuromuscular junction (NMJ; black dots). Because the myofibers are usually ruptured on either side of the row of the NMJs of the adjacent fibers, the adjunctional stumps of fibers 1 and 3 to 5 on the "ad" side (right) remain innervated, whereas their abjunctional stumps on the "ab" side (left) become denervated. Even the adjunctional stump of fiber 3 has become denervated because its NMJ is located in the RZ. Reinnervation of the abjunctional stumps occurs via penetration of new axon sprouts through the scar of the CZ and the formation of the new NMJs (shown here 1 sprout and NMJ [white dot]). Fiber 3 becomes reinnervated when regeneration in the adjunctional RZ takes place.

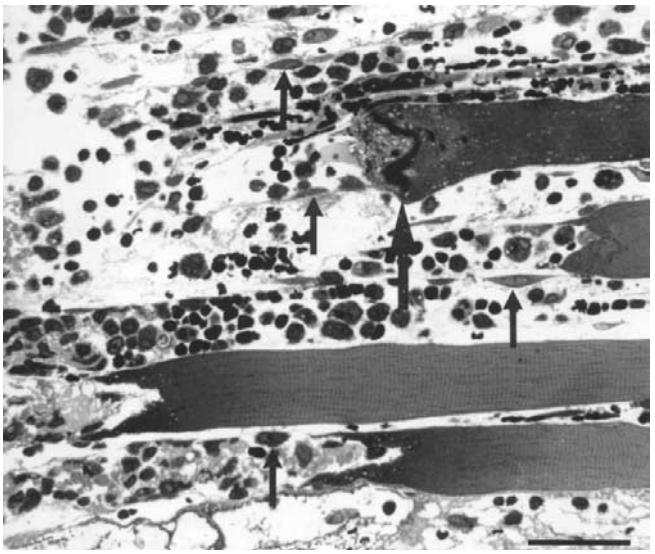


Figure 4. A semi-thin epon section of a ruptured muscle 2 days after a shearing injury. The surviving parts of the 4 myofibers are sharply demarcated from the necrotized segments, which have become transformed into basal lamina cylinders containing macrophages (round cells) phagocytosing the necrotic debris. The contraction band (thick arrow) that halts the propagation of necrosis is still clearly visible in fiber 1 but has disappeared in fibers 2 to 4. The regeneration process has already begun, as evidenced by the existence of myoblasts within the basal lamina cylinders (thin arrows). Toluidine blue. Bar 50 μm.

borne inflammatory cells gain direct access to the injury site (Figures 2 and 4).[154,156] The beginning of the inflammatory reaction is later "amplified" as the satellite cells and the necrotized parts of the myofibers release various substances (wound hormones), which serve as chemoattractants enhancing the extravasation of the inflammatory cells.[30,57,154] Within the injured muscle, there are macrophages and fibroblasts that are activated and produce additional chemotactic signals (eg, growth factors, cytokines, and chemokines) for the circulating inflammatory cells.[¶] In addition to these growth factors produced de novo, the majority of tissues contain growth factors stored in an inactive form in their ECMs to be used when acutely needed, for example, in repair of an injury.[124] These "storage" growth factors are produced by normal resident cells and inactivated by their strong adherence to proteoglycans and other constituents of the ECM.[124] However, in the event of tissue damage, the disruption of normal tissue integrity results in the activation/release of these ECM-bound growth factors, and they start to direct the repair process.[124]

[¶]References 24, 25, 57, 129, 143, 154, 162, 167.

Concerning the growth factors and cytokines, there is direct evidence that tumor necrosis factor–α (TNF-α) has a physiological role in the regeneration of the injured skeletal muscle, as the inhibition of its activity during the healing results in a slight deficit in the strength of the recovering skeletal muscle.[161] Furthermore, a large number of growth factors and cytokines, such as the members of fibroblast growth factor (FGF), insulin-like growth factor (IGF), and transforming growth factor–β (TGF-β) families; hepatocyte growth factor (HGF); and interleukin-1β (IL-1β) and IL-6, are known to be expressed in the injured skeletal muscle,[#] and it is likely that several other growth factors such as the platelet-derived growth factor are also expressed in the injured muscle,[23,109] at least their expression can be induced in the skeletal muscle by such physiological stimuli (that actually causes micro-traumas) as external stretching or mechanical loading.[23,122] Considering that these growth factors are potent mitogenic activators for numerous different cells, they are also likely to be involved in the activation of the regeneration of the injured muscle cells.[16,23,29] A number of these growth factors, such as FGFs, IGF-1, IGF-2, TGF-β, HGF, TNF-α, and IL-6, are potential activators of myogenic precursor cell (mpc; satellite cell) proliferation.[29] Some of them are also powerful stimulators for mpc differentiation and the

[#]References 7, 22, 23, 29, 33, 52, 55, 143, 147, 167.

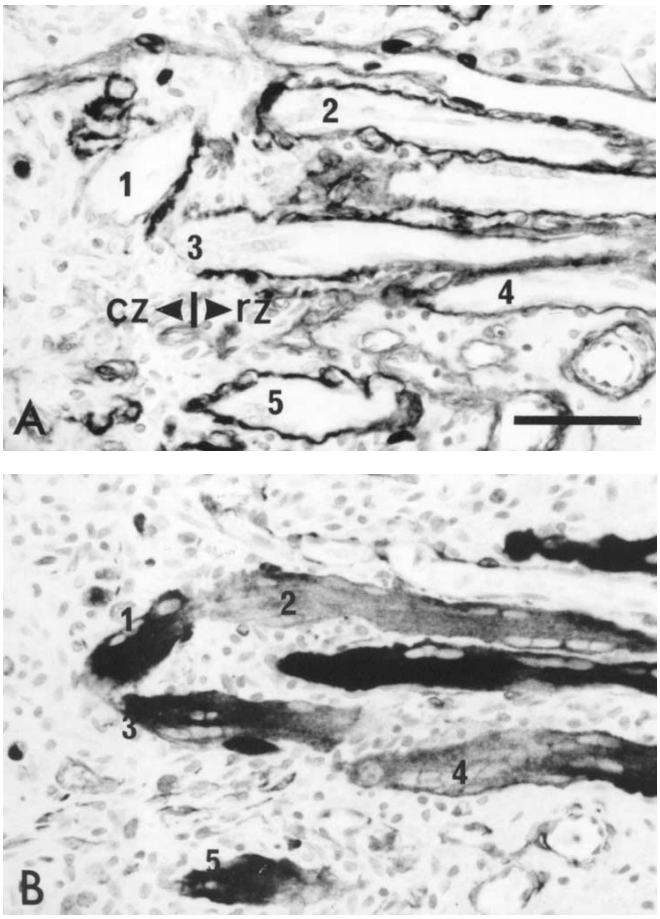


Figure 5. By day 5 after a contusion injury, the basal lamina cylinders of the regeneration zone (RZ), which are shown by immunopositivity for laminin (A), have become filled with strongly desmin immunopositive myotubes with chains of nuclei as shown (B). The open orifice of the ruptured basal lamina at the interface between the central zone (CZ) and RZ (see also Figure 1) is best visible in fiber 3. Consecutive sections with corresponding fibers are indicated by numbers. Hematoxylin counterstain. Bar 50 μm.

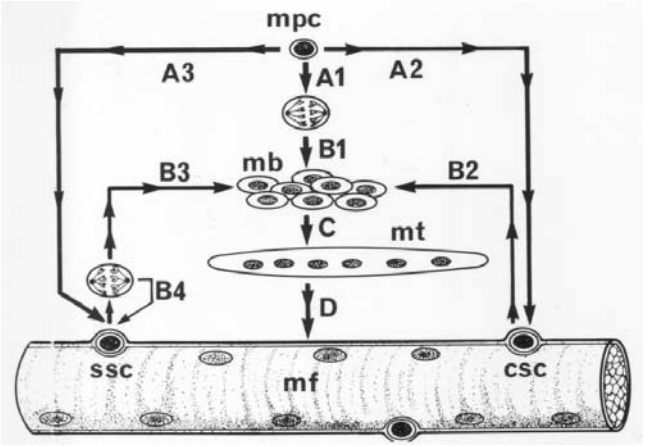


Figure 6. A schematic presentation of fetal development (A1-B1-C-D) and regeneration of myofibers via the activation of satellite cells (A2-B2-C-D or A3-B3-C-D). Satellite cells have been set aside underneath the basal lamina during the fetal development (A2 and A3) to be used in growth and repair. After injury, the committed satellite cells (csc) immediately begin differentiation into myoblasts (mb) without prior cell division (B2), while the stem satellite cells (ssc) first divide, and only then one of the daughter cells differentiates into a myoblast (B3), whereas the other replenishes the pool of stem satellite cells (B4). Myoblasts fuse into myotubes (mt), which then grow and mature into myofibers, the sarcoplasm of which becomes filled with contractile filamentous proteins organized as myofibrils and with the myonuclei located subsarcolemmally. mpc, myogenic precursor cell.

fusion of myotubes into multinucleated mature myofibers later during the regeneration process.[16,23,29]

In the very acute phase after an injury to a skeletal muscle, polymorphonuclear leukocytes are the most abundant cells at the injury site,[18,19,62,135,142] but within the first day, they are being replaced by monocytes. According to the basic principles of inflammation, these monocytes are eventually transformed into macrophages that then actively engage in the proteolysis and phagocytosis of the necrotic material by the release of lysosomal enzymes.[13,43,62,154] Macrophage phagocytosis is a process remarkably specific to the necrotic material, as the preserved cylinders of the basal laminas surrounding the necrotized parts of the injured myofibers survive (are left intact by) the attack of the macrophages and consequently serve as scaffolds inside which the viable satellite cells begin the formation of new myofibers[52,60,62] (Figure 4). As a fascinating demonstration of the unbelievable specificity and high coordination of this process, the magrophages, while phagocytosing the necrotic debris surrounding the satellite cells, simultaneously send soluble survival factors for these regenerative cells.[30]

REPAIR AND REMODELING PHASE

Once the destruction phase has subsided, the actual repair of the injured muscle begins with 2 concomitant—simultaneously supportive and competitive—processes, the regeneration of the disrupted myofibers (nerves) and the formation of a connective tissue scar (Figure 2). A balanced progression of both of these processes is a prerequisite for optimal recovery of the contractile function of the muscle.[62,88]

Regeneration of Myofibers

Although the myofibers are generally considered to be irreversibly postmitotic, the marked regenerative capacity of the skeletal muscle is guaranteed by an intrinsic mechanism that restores the injured contractile apparatus. Accordingly, a pool of undifferentiated reserve cells called the satellite cells are set aside, underneath the basal lamina of each individual myofiber[60,88,125] (Figures 4 to 6), dur-

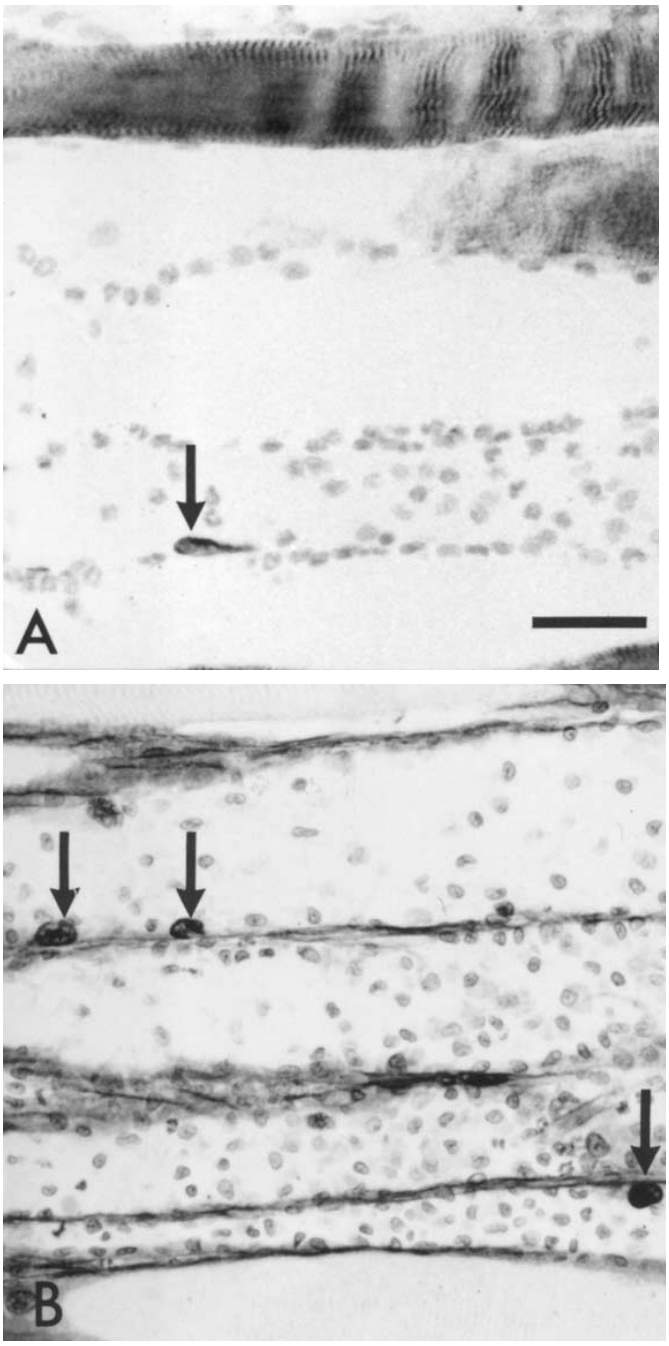


Figure 7. A, a myoblast (arrow) with desmin-positive sarcoplasm is already visible within the basal lamina cylinder of the necrotized part of the sarcoplasm 21 hours after the injury, indicating that it must have differentiated from a committed satellite cell (antidesmin and hematoxylin counterstain). B, the first mitoses of the stem satellite cells (arrows) are seen approximately 24 hours after the injury, visualized here by immunostaining for bromodeoxyuridine (a thymidine analog) incorporated in nuclear DNA during the S-phase of the cell cycle (antibromodeoxyuridine and hematoxylin counterstain). Bar 30 μm.

ing the fetal development. In response to injury, these cells first proliferate, then differentiate into myoblasts, and finally join with each other to form multinucleated myotubes[60] (Figure 6). The newly formed multinucleated myotubes then fuse with the part of the injured myofiber that has survived the initial trauma.[60] Eventually, the regenerating parts of the myofibers acquire their mature form with normal cross-striations and peripherally located myonuclei[60] (Figure 6). Interestingly, in response to very mild injury (a single, eccentric stretch-induced injury), the satellite cells respond immediately by starting to proliferate, but because of the mildness of the injury and rapid, "intrinsic" recovery of the injured myofibers, the satellite cell activation halts before the myoblasts arise.[2]

In mature skeletal muscle, there are (at least) 2 major populations of satellite cells[66,88,123,125,130,170] (Figures 6 and 7). The "classic" satellite cells, those residing beneath the muscle fiber basal lamina, can be divided into committed satellite cells, which are ready to begin differentiation to myoblasts immediately after the muscle injury (Figure 7A), and stem satellite cells, which first undergo cell division(s) before differentiation[88,125,170] (Figure 7B). Through this cell division (proliferation), the stem population replenishes the reserve of satellite cells for the possible future demands of regeneration[125,170] (Figure 6). Among this population of satellite cells, there appears to be a subpopulation of cells that are capable of differentiating beyond myogenic lineages not only into different mesenchymal lineages[137] but also into the neural or endothelial ones.[66,123]

Until recently, the satellite cells were presumed to be the only source of myonuclei in muscle repair.[29] However, recent findings have demonstrated the presence of 2 different populations of multipotential stem cells that can contribute to the regeneration of injured skeletal muscle: nonmuscle resident stem cells and muscle resident stem cells.[29] Progenitor cells isolated from bone marrow (BM), the neuronal compartment, and various mesenchymal tissues can differentiate into a myogenic lineage. The BM-derived cells not only contribute to the regenerating myofibers in injured skeletal muscle but also replenish the satellite cell pool in the injured skeletal muscle.[94] However, it is worth noting that the frequency at which these events occur seems to be very low (even in the injury) when compared with the number of regenerating myoblasts derived from the satellite cells.[53,94] Thus, it is debatable whether the stem cells make a significant contribution to the regeneration of the injured skeletal muscle at all.[53]

In addition to the classic satellite cells residing underneath the basal lamina, there is another distinct population of muscle stem cells located extralaminally within the connective tissue of the skeletal muscle.[40] In response to injury to the skeletal muscle, these cells readily give rise to determined myoblasts and differentiate to myotubes.[29]

After the cylinders of the old basal lamina have been filled with the regenerating myofibers (Figure 5), the myofibers further extend through the opening on the basal lamina toward the connective tissue scar that has formed

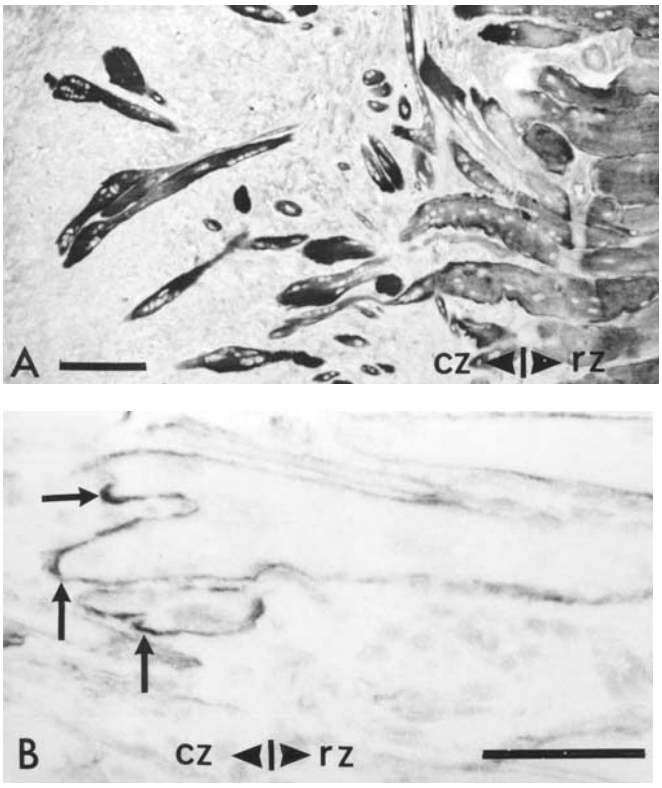


Figure 8. A, 7 days after a contusion injury, the tips of the regenerating muscle cells extend out of the orifices of the old basal lamina tubes and begin to penetrate into the scar tissue in the central zone (antidesmin and hematoxylin counterstain; bar 100 μm). B, this penetration is soon halted by the formation of new mini–myotendinous junctions at the tips of the myofibers, whereby the adhesion of myofibers to the extracellular matrix becomes reestablished. These new myotendinous junctions appear as accentuated immunostaining with antibody to the muscle-specific α7 chain of adhesion molecule integrin α7β1 (arrows) (anti-integrin α7 and hematoxylin counterstain; bar 30 μm). CZ, central zone; RZ, regeneration zone.

between the survived stumps of the myofiber[62,88] (Figure 8A). On both sides of the connective tissue scar, the myofibers of the survived muscle stumps form multiple branches while trying to pierce through the scar separating them.[62] However, after managing to extend only for a relatively short distance, the branches begin to adhere to the connective tissue with the tips at their ends, forming mini-MTJs with the scar (Figure 8B). With time, the intervening scar progressively diminishes in size, bringing the stumps in closer adherence to each other,[157] but it is still unknown whether the stumps of the transected myofibers from the opposing sides of the scar will ultimately ever fuse with each other or whether some form of connective tissue septum remains between them.[1,157]

It has been shown that the regenerative capacity of skeletal muscle in response to injury is significantly reduced with age.[71] This diminished capacity is apparently not attributable to a decrease in the number or activity of the satellite cells[71] but rather to an overall decline in the regenerative capacity of the aged muscle, as each phase of the repair process seems to slow down and deteriorate with age.[71]

Formation of the Connective Tissue Scar

Immediately after an injury to the skeletal muscle, a gap formed between the ruptured muscle fibers is filled with a hematoma. Within the first day, inflammatory cells including phagocytes invade the hematoma and begin to dispose the blood clot.[24,62,154] Blood-derived fibrin and fibronectin cross-link to form early granulation tissue, an initial ECM that acts as a scaffold and anchorage site for the invading fibroblasts[62] (some of the fibroblasts in granulation tissue may also be derived from the myogenic cells).[102] More important, this newly formed tissue provides the wound tissue with the initial strength to withstand the contraction forces applied to it.[63,97-99] Fibroblasts then start synthesizing the proteins and proteoglycans of the ECM to restore the integrity of the connective tissue framework.[51,63,97-99]

Among the first of these synthesized ECM proteins are tenascin-C (TN-C) and fibronectin,[51,61,63,97] which first turns into multimeric fibrils and then forms the superfibronectin with greatly enhanced adhesive properties.[112,163] Both fibronectin and TN-C possess elastic properties, being capable of stretching to several times their resting length by the mechanical loading applied to tissue, and they are thought to provide strength and early elasticity to the early granulation tissue in injured skeletal muscle.[77,78,80] The expression of fibronectin is soon followed by that of type III collagen,[16,51,63,97-99] but the production of type I collagen is only initiated a couple of days later, although it remains elevated for several weeks.[16,63,97-100,167] The initially large granulation tissue (scar intervening the surviving muscle stumps) condenses very efficiently into a remarkably small connective tissue mass composed mainly of type I collagen.[62,67,73,97-100] Despite the prevailing proposals that general fibrosis occurs in the healing skeletal muscle,[59] the amount of intramuscular connective tissue is not increased in the injured skeletal muscle unless the muscle is completely immobilized for a substantial period of time.[67,73,97]

The connective tissue scar produced at the injury site is the weakest point of the injured skeletal muscle early after trauma,[62,85] but its tensile strength increases considerably with the production of type I collagen.[85,97,100] The mechanical stability of the collagen, in turn, is attributable to the formation of intermolecular cross-links during the maturation of the scar tissue.[100] Approximately 10 days after the trauma, the maturation of the scar has reached the point at which it no longer is the weakest link of the injured muscle, but, rather, if loaded to failure, the rupture usually occurs within the muscle tissue adjacent to the newly formed mini-MTJs between the regenerated myofibers and the scar tissue.[67,69,85] However, a relatively long time is still needed until the strength of the muscle is completely restored to the preinjury level.[67,69,85]

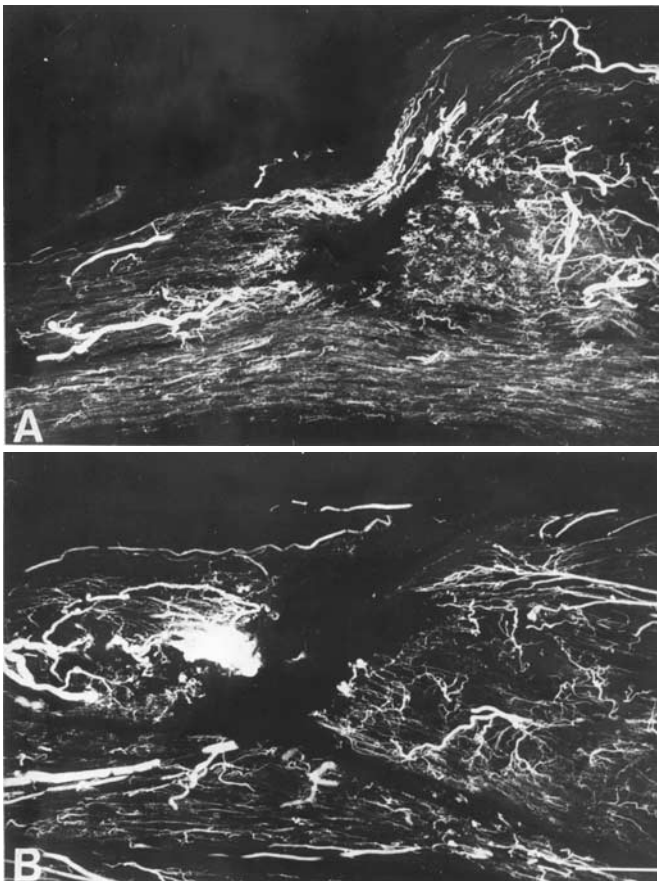


Figure 9. Capillary ingrowth to the injured skeletal muscle 5 days after a contusion injury treated by either mobilization (A) or immobilization (B). In the mobilized muscle (A), an intensive ingrowth of new capillaries from all borders of the surviving muscle surrounding the injury toward the nonvascularized center of the injury is seen, whereas in the immobilized muscle (B), the capillary ingrowth to the injured area is almost completely negligible and a large intramuscular hematoma is still visible (on the left side of the wound). Micro-angiograph; bar 300 μm.

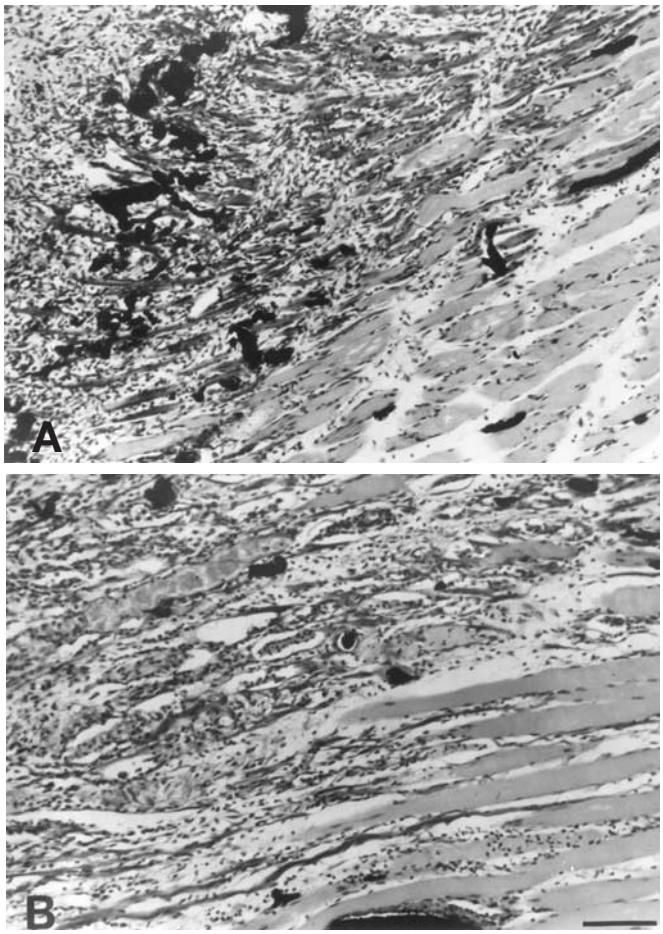


Figure 10. A, numerous young, regenerating myotubes are seen in close connection with the capillaries in the regeneration zone of the skeletal muscle injury treated by mobilization 5 days after a contusion injury. B, only a few small myotubes as well as a few capillaries are found in the granulation tissue of the regenerating zone of the injured skeletal muscle when the muscle is treated by immobilization. Van Gieson hematoxylin counterstain. Bar 150 μm.

Although a great majority of the injuries to the skeletal muscle heal without formation of the functionally disabling fibrous scar, the proliferation of fibroblasts can sometimes be excessive, resulting in the formation of a dense scar tissue within the injured muscle. In such cases, usually associated with major muscle trauma or particularly with reruptures, the scar can create a mechanical barrier that considerably delays or even completely restricts the regeneration of the myofibers across the injury gap.[67,69] Experimental studies have recently provided interesting insights pertinent to the scar formation in the injured skeletal muscle; it was shown that direct application of either small leucine-rich proteoglycan (SLRP) decorin or antifibrotic agent suramin or γ-interferon inhibits the scar formation in the injured skeletal muscle.[28,44,47] Decorin, suramin, and γ-interferon are all specific inhibitors of TGF-β,[28,52,56,166] a growth factor that is believed to be responsible for the scar formation during the wound/skeletal muscle repair. In addition to the inhibition of TGF-β, decorin and other SLRPs can not only bind to different collagens but also regulate the fibrillogenesis and assembly of type I collagen fibrils.[35,45,115]

Vascularization of the Injured Muscle

A vital process in the regeneration of an injured muscle is the vascularization of the injured area.[68,82,139] The restoration of vascular supply to the injured area is the first sign of regeneration and a prerequisite for the subsequent morphological and functional recovery of the injured muscle[68] (Figures 9A and 10A). The new capillaries sprout from the surviving trunks of the blood vessels toward the center of the injured area[68] to provide the area with an adequate supply of oxygen, subsequently enabling aerobic

energy metabolism for the regenerating myofibers[82] (Figure 9A). Young myotubes have few mitochondria and only a moderate capacity for aerobic metabolism but have clearly increased anaerobic metabolism.[75] However, during the final stages of regeneration, aerobic metabolism constitutes the principal energy pathway for the multinucleated myofibers.[75] This process also provides a plausible explanation as to why the regeneration of the myofibers does not progress beyond the newly formed thin myotube stage unless a sufficient capillary ingrowth has ensured the required supply of oxygen for the aerobic metabolism.[68,75]

Regeneration of Intramuscular Nerves

Similar to the vascularization process, the regeneration of the skeletal muscle can also be halted by the unsuccessful regeneration of the intramuscular nerves[64,126,158,159] (Figure 3). Myofiber regeneration continues to the myotube phase even in the absence of innervation but atrophy ensues if reinnervation is not accomplished[126] (Figure 3). In case of neurogenic denervation (rupture of the axon), the reinnervation requires the regrowth of a new axon distal to the rupture. Because axons are usually ruptured within or next to the muscle, the nerve-muscle contact is fairly rapidly reestablished.[88]

Adhesion of Myofibers to the ECM

When myofibers are breached, the continuity of the tendon-muscle-tendon unit is disrupted at the rupture site, and the contractile force cannot be transmitted across the gap formed between the ruptured stumps. Thus, the stumps are simply pulled further apart during contraction. The ends of the regenerating myofibers, attempting to pierce through the scar tissue, maintain a growth cone appearance for a relatively long period of time during regeneration,[61,62] a time period during which the ends cannot firmly attach to the scar. Instead, the regenerating myofibers reinforce their adhesion to the ECM on their lateral aspects in both the intact and regenerating parts of the myofibers[3,84,141] (Figure 11). This reinforced lateral adhesion reduces both the movement of the stumps and the pull on the still-fragile scar, reducing the risk of rerupture while allowing some use of the injured muscle before the healing is completed.[83,86,87] Quite interestingly, it actually appears that mechanical stress is a prerequisite for the lateral adhesion, as recent experimental studies have shown that the phenomenon does not occur in the absence of mechanical stress.[86]

Later during the regeneration process, the strong terminal adhesions consisting of the same adhesion molecules as the normal MTJs (ie, clusters of integrin- and dystrophin-associated molecules) are established at the ends of the stumps[83,84,86,87,140] (Figures 11 and 8B). Accordingly, the original (preinjury) tendon-myofiber-tendon unit becomes replaced by 2 successive tendon-myofiber-mini-MTJ units separated by the scar. These 2 successive units contract synchronously, as both become reinnervated by the same nerve.[126] On the ECM side of the new mini-MTJs, elastic and adhesive molecules are heavily expressed, absorbing the forces created by muscle contractions.[61,80] Having reestablished firm terminal adhesion through these mini-MTJs, the myofibers no longer need the reinforced lateral adhesion, and accordingly, the high expression of integrins on the lateral sarcolemma decreases.[84] The interposed scar

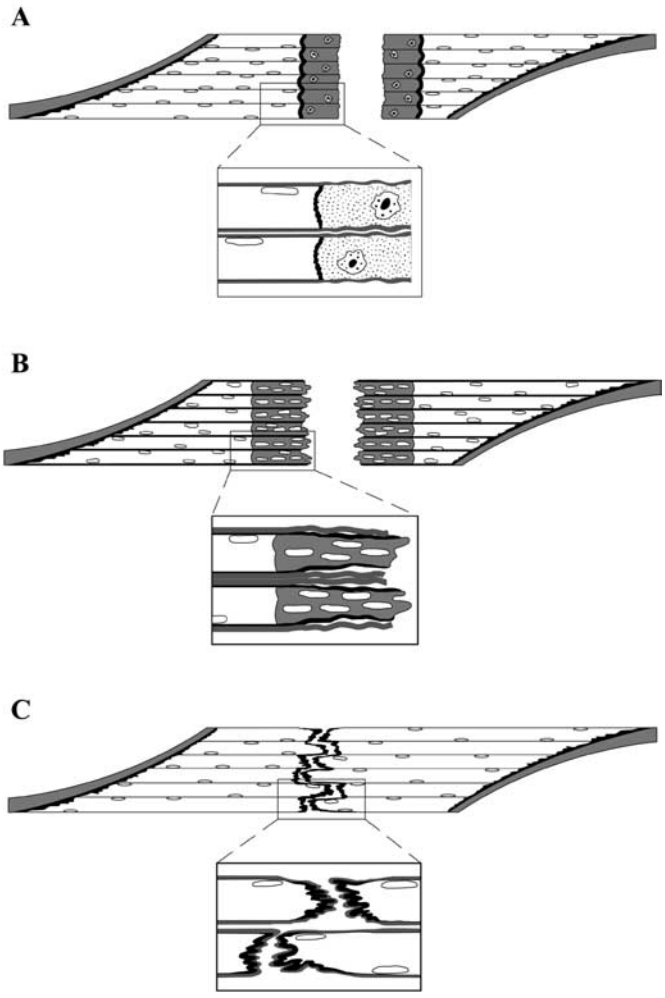
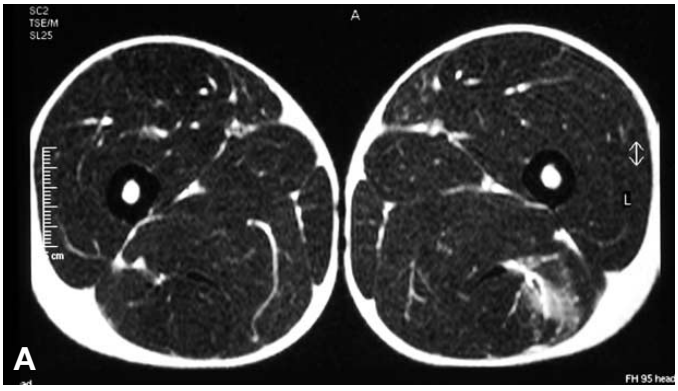
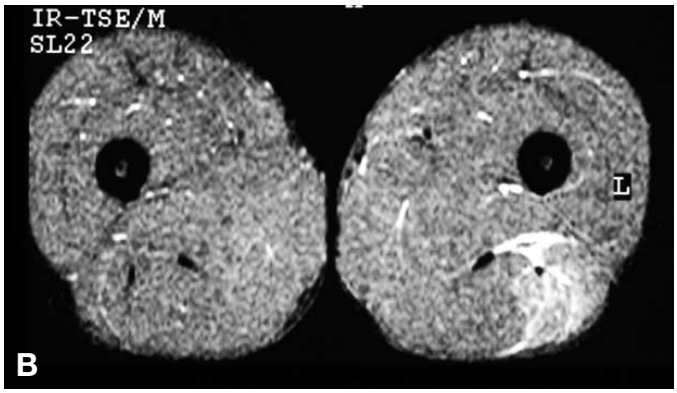
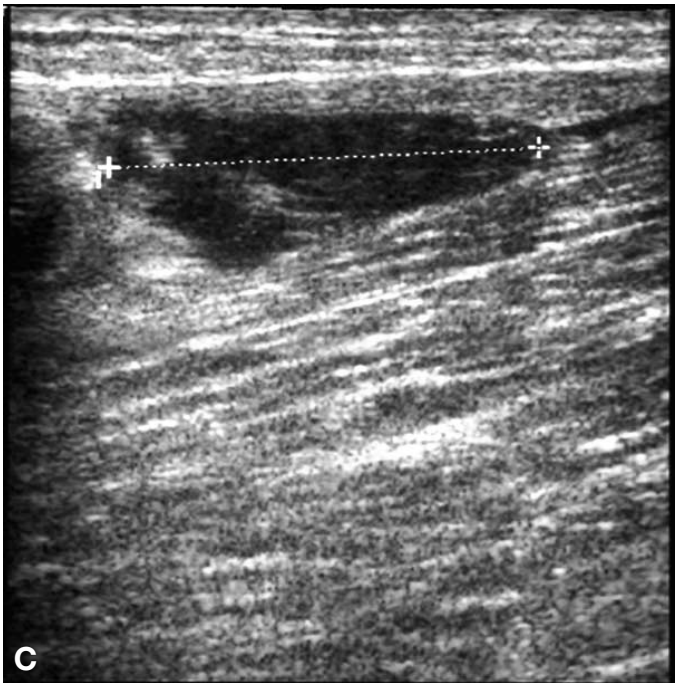


Figure 11. A, in the beginning of the healing process of the injured skeletal muscle, the expression of cell adhesion molecule integrin ($\alpha7\beta1$) is enriched at the end of the regenerating part of the injured muscle fibers, whereas only minor amounts are present on the lateral aspects of the myofiber. B, a dramatic increase in the expression of the $\alpha7\beta1$ integrin takes place along the lateral aspect of the plasma membrane in both the intact and regenerating parts of the injured myofibers when the regenerating muscle fibers pierce into wound tissue. Thus, the $\alpha7\beta1$ provides stability for the regenerating muscle fibers that lack adhesion at their ends. C, the expression of the $\alpha7\beta1$ integrin returns to normal level on the lateral sarcolemma with simultaneous redistribution of $\alpha7\beta1$ integrin to the ends of the regenerating myofibers when they form new myotendinous junctions and adhere to the scar.







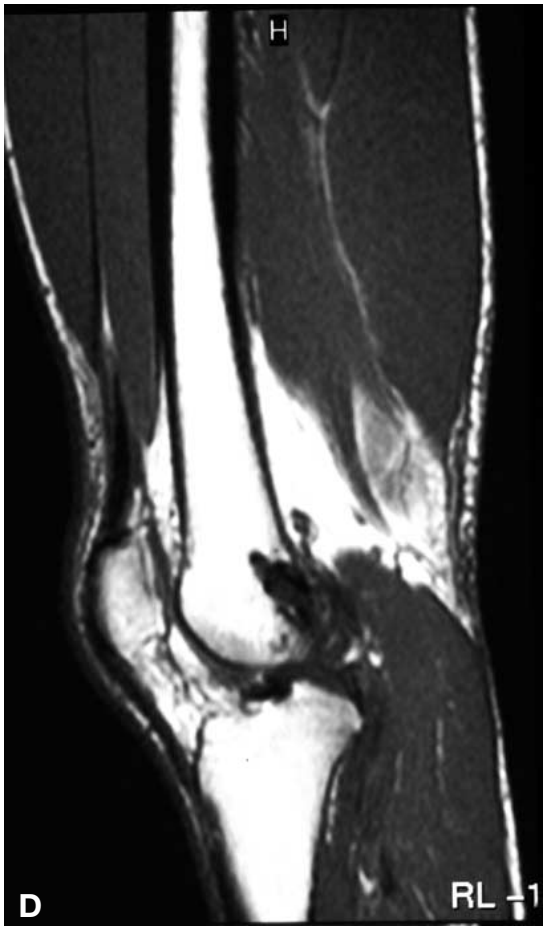


Figure 12. The (A) T2-weighted and (B) fat-suppressed (short T1 inversion recovery) MRI image of an acute (approximately 7-day-old) grade I muscle strain in the left hamstring muscle; this injury was not detectable by ultrasonography. A demonstration of corresponding longitudinal ultrasonography (C) and MR (D) images of an acute tear of the hamstring muscle insertion.

gradually diminishes in size, thereby bringing the stumps closer together until the myofibers finally become interlaced, although most likely not reunited[84,85,157] (Figure 11C).

CLINICAL CLASSIFICATION OF MUSCLE INJURIES

The clinical picture of a muscle injury—strain, contusion, or laceration—depends on the severity of the injury and the nature of the hematoma. The intramuscular blood vessels are torn relatively easily as a result of trauma, leading to either intramuscular or intermuscular hematoma. In case of the intramuscular hematoma, the extravasation of blood within the intact muscle fascia results in increased intramuscular pressure, which subsequently compresses and eventually limits the size of the hematoma. In contrast, an intermuscular hematoma develops if the fascia surrounding the muscle is torn and the extravasated blood has free access to spread into the interstitial and interfascial spaces without a significant increase in the pressure within the muscle.

The current classification of muscle injuries identifies mild, moderate, and severe injuries based on the clinical impairment they bring about. Mild (first-degree)

strain/contusion represents a tear of only a few muscle fibers with minor swelling and discomfort accompanied by no or only minimal loss of strength and restriction of the movements. Moderate (second-degree) strain/contusion, in turn, is a greater damage of the muscle with a clear loss in function (ability to contract), whereas a tear extending across the entire cross section of the muscle and, thus, resulting in a virtually complete loss of muscle function is termed severe (third-degree) strain/contusion.

DIAGNOSIS OF MUSCLE INJURIES

The diagnosis of a muscle injury begins with a careful history of the occurrence of the trauma, followed by clinical examination consisting of inspection and palpation of the involved muscles, as well as testing of the function of the injured muscles both without and with external resistance. The diagnosis is easy when a typical history of muscle contusion or strain is accompanied by objective evidence of swelling and/or ecchymosis distal to the lesion. Hematomas that are small in size and those deep within the muscle belly can be more difficult to diagnose clinically, but the imaging modalities (ultrasonography, CT, or MRI) provide useful means to more precisely verify and determine the injury[8,39,138,150] (Figure 12). Ultrasonography has traditionally been considered the method of choice for clinical diagnosis of muscle injuries, as it is relatively inexpensive in nature. However, it has the clear disadvantage of being highly dependent on the experience of the radiologist, and accordingly, MRI has more recently replaced ultrasonography in the imaging of many musculoskeletal disorders.[39,138] Concerning muscle injuries in particular, MRI can accurately confirm/rule out the existence of muscle injury and provides a very detailed characterization of the lesion (Figure 12), even to the extent of being considered somewhat oversensitive at times. To summarize, the clinical diagnosis of muscle injury is sufficient in most cases, but ultrasonography can be considered a valid first-line tool if a more exact characterization of the injury is desired. Magnetic resonance imaging, in turn, should be preferred if a clear discrepancy exists between the patient's symptoms, the physician's findings, and/or the ultrasonography, particularly in injuries near and/or at the groin area or close to the MTJ,[39,73] where MRI has shown its superiority over ultrasonography.

THE TREATMENT PRINCIPLES OF MUSCLE INJURIES

The current treatment principles of injured skeletal muscle lack firm scientific basis. In the following section, we will review the existing literature on different treatment modalities for muscle injuries. We have attempted to elucidate the theoretical basis of some of the basic principles in the treatment of injured skeletal muscle, to briefly review the various specific modalities currently in use in clinical practice, and to provide our clinical recommendations accompanied by a rationale for the suggested actions.

IMMOBILIZATION AND REMOBILIZATION ON MUSCLE HEALING

Early mobilization was first recommended for the acute treatment of muscle trauma by Dr Woodard in 1954,[165] largely based on his vast personal experience in treating injured athletes. Today, we have quite a considerable amount of scientific, mostly experimental evidence to support this treatment approach of muscle injuries.** For example, it has been shown that early mobilization induces more rapid and intensive capillary ingrowth into the injured area, better regeneration of muscle fibers, and more parallel orientation of the regenerating myofibers in comparison to immobilization, the previously preferred treatment for injured muscle (Figures 9 and 10).[67-69,74,75] The positive effects of early mobilization on the regeneration of the injured skeletal muscle are not only limited to histologic changes, as it has been shown that the biomechanical strength of the injured muscle returns to the level of uninjured muscle more rapidly using active mobilization than if the muscle is immobilized after the trauma.[69]

However, the most appropriate mobilization of an injured muscle is not as straightforward as one might assume. Experimental studies have also shown that if active mobilization—or even a slight use of the injured muscle—is begun immediately after the injury, a larger connective tissue scar ensues, and the initial penetration of muscle fibers through the connective tissue scar appears to be impaired in comparison to immobilized muscle.[67] In addition, reruptures at the site of the original muscle trauma are common if active mobilization is begun immediately after the injury.[67-69,74,97] In fact, somewhat paradoxically, it has actually been shown that by placing the injured muscle to rest (cast immobilization in rats) for the first couple of days after the injury, the excessive scar formation and reruptures at the injury site can be best prevented.[67-69,73-75,93,97] Immobilization appears to provide the new granulation tissue with the needed tensile strength to withstand the forces created by muscle contractions.[69,73,97]

Although immobilization has been shown to result in beneficial effects in the early phase of muscle regeneration, it also has several clinically undesired effects. For example, inactivity has been shown to be associated with a significant atrophy of the healthy muscle fibers, excessive deposition of connective tissue within the muscle tissue, and a substantially retarded recovery of the strength of the injured skeletal muscle throughout the immobilization period.†† If immobilization is continued past the acute phase (first few days) of muscle regeneration, the deleterious effects become particularly evident during the remodeling phase of muscle healing[67] (Figure 10). In summary, a short period of immobilization after muscle injury is beneficial, but it should be limited only to the first few days after the injury. This rest period allows the scar tissue connecting the injured muscle stumps to gain the required

**References 21, 67-69, 74-76, 88, 90, 92, 93, 97.
††References 67-69, 73-75, 79, 88, 90, 93, 97.

strength to withstand the contraction-induced forces applied on it without a rerupture, but being restricted to the first few days only, the adverse effects of immobility per se can be limited to a minimum.[69,73]

Experimentally, it has been shown that by day 10 after the trauma, the muscles tested in tension showed failure in the intact part of the muscle,[84] suggesting that the tensile strength of the connective tissue scar becomes greater than that of the muscle tissue at that point. However, the active use of the injured muscle can and should be carefully started before this point, as there is also experimental data showing that beginning active mobilization after the short period of immobilization enhances the penetration of muscle fibers through the connective tissue scar, limits the size of the permanent scar, facilitates the proper alignment of the regenerating muscle fibers, and helps in regaining the tensile strength of the injured muscle.[67-69,73-75,88,97]

Largely based on these experimental findings, we have adopted the following practice for the treatment of our athletes with acute muscle injuries. The required relative immobility can be achieved simply by applying a firm adhesive taping or the like over the injured muscle, so a cast is naturally not needed. We highly recommend the use of crutches for the athletes with the most severe lower extremity muscle injuries, as well as when the injury is located at a site where adequate immobilization is otherwise difficult to attain, such as the groin area.[92] We also instruct the athlete to move very carefully for the first 3 to 7 days after the injury to prevent the injured muscle from stretching. After this period of relative immobility, more active use of the injured muscle can be started gradually within the limits of pain.

IMMEDIATE TREATMENT: THE REST, ICE (COLD), COMPRESSION, AND ELEVATION PRINCIPLE

The immediate treatment of the injured skeletal muscle, or any soft tissue injury for that matter, is known as the rest, ice (cold), compression, and elevation (RICE) principle. The overall justification for the use of the RICE principle is very practical, as all 4 means aim to minimize bleeding into the injury site. It needs to be stressed that there is not a single, randomized clinical trial to prove the effectiveness of the RICE principle in the treatment of soft tissue injury.[17] However, there is scientific proof for the appropriateness of the distinct components of the concept, the evidence being derived from experimental studies.

The most persuasive proof for the use of rest has been obtained from studies on the effects of immobilization on muscle healing.[73] By placing the injured extremity to rest immediately after the trauma, one can prevent further retraction of the ruptured muscle stumps (the formation of a large gap within the muscle) as well as reduce the size of the hematoma and, subsequently, the size of the connective tissue scar.[73] Regarding the use of ice, it has been shown that the early use of cryotherapy is associated with a significantly smaller hematoma between the ruptured myofiber stumps, less inflammation, and somewhat accelerated early regeneration.[38,65] Although compression reduces the intramuscular blood flow to the injured area,[88,148] it is debatable whether compression applied immediately after the injury actually accelerates the healing of the injured skeletal muscle.[151] However, according to the prevailing belief, it is recommended that the combination of ice (cryotherapy) and compression be applied in shifts of 15 to 20 minutes in duration, repeated at intervals of 30 to 60 minutes, as this kind of protocol has been shown to result in a 3° to 7° C decrease in the intramuscular temperature and a 50% reduction in the intramuscular blood flow.[148,149] Finally, concerning the last component of the RICE principle, elevation, the rationale for its use is based on the basic principles of physiology and traumatology; the elevation of an injured extremity above the level of the heart results in a decrease in hydrostatic pressure and, subsequently, reduces the accumulation of interstitial fluid.

TREATMENT AFTER 3 TO 5 DAYS

If the acute phases after the injury have passed uneventfully and the recovery of the injured limb seems to be progressing favorably, the more active treatment of the injured muscle should be started gradually using the following specific exercises:

1. Isometric training (ie, muscle contractions in which the length of the muscle remains constant and the tension changes) should be started first without a resisting load/counterload and then later with increased loads. Special attention should be paid to ensure that all of these isometric exercises are performed only within the limits of pain.
2. Isotonic training (ie, the muscle length changes and the tension remains constant during muscle contraction) can be started when isometric training can be performed pain free with resisting loads. Similar to isometric training, isotonic exercises should be first carried out without a resisting load/counterload, and the loading should then be progressively increased.
3. Isokinetic, dynamic training with minimal load should be started once the 2 above-mentioned exercises can be performed without pain.

The local application of heat or contrast treatment (cold and heat treatment in succession) may be of value, accompanied by careful passive and active stretching of the affected muscle within the limits of pain. It is of particular importance to note that all physical rehabilitation activities should always start with an adequate warming up of the injured muscle,[103,116,132,133] as it has been shown to reduce muscle viscosity and relax muscles neurally. Furthermore, the stimulated, warm muscles absorb more energy than unstimulated muscles do and can thus better withstand loading.[116,132,133] When warming up is combined with stretching, the elasticity of muscle is improved,[132,133] further increasing the capacity of the injured muscle to

resist a new tear (rerupture).[132,133] The other purpose of stretching is to distend the maturing scar during a phase in which it is still plastic but already has the required strength to prevent a functionally disabling retraction of the muscle stumps. Painless elongation of the scar can be achieved by gradual stretching, beginning with shifts of 10 to 15 seconds at a time and then proceeding up to a period of 1 minute. Stretching should also involve repeated stretches of the same muscle because repeated elongation has been shown to decrease the (counter)resistance of the muscle to stretching.[103]

However, if the symptoms caused by the injured muscle fail to improve 3 to 5 days after the trauma, this is the stage at which it is necessary to reconsider the existence of an intramuscular hematoma or extensive tissue damage that might require special attention. Accordingly, a thorough clinical reexamination should be carried out with special emphasis on the contractile status of the injured muscle, which ultimately dictates the need for surgical intervention. The imaging modalities (ultrasonography or especially MRI) are highly recommended under these circumstances.[146] The puncture and aspiration of the injured area (if fluctuation is present) are among the procedures that are sometimes required.

OPERATIVE TREATMENT

One should exercise extreme caution in considering surgical intervention in the treatment of muscle injuries, as a properly executed nonoperative treatment results in a good outcome in virtually all cases. In fact, the phrase *muscle injuries do heal conservatively* could be used as a guiding principle in the treatment of muscle traumas. Having said that, there are certain highly specific indications in which surgical intervention might actually be beneficial. These indications include an athlete with a large intramuscular hematoma(s), a complete (III degree) strain or tear of a muscle with few or no agonist muscles, or a partial (II degree) strain if more than half of the muscle belly is torn.[6,92] Also, surgical intervention should be considered if a patient complains of persisting extension pain (duration, >4-6 months) in a previously injured muscle, particularly if the pain is accompanied by a clear extension deficit. In this particular case, one has to suspect the formation of scar adhesions restricting the movement of the muscle at the site of the injury, a phenomenon that often requires surgical deliberation of the adhesions.

If surgery is indeed warranted in the treatment of an acute skeletal muscle injury, the following general principles are recommended: the entire hematoma and all necrotic tissue should be carefully removed from the injured area. One should not attempt to reattach the ruptured stumps of the muscle to each other via sutures unless the sutures can be placed through a fascia overlying the muscle. Sutures placed solely through myofibers possess virtually no strength and will only pierce through the muscle tissue. Loop-type sutures should be placed very loosely through the fascia, as attempts to overtighten them will only cause them to pierce through the myofibers beneath the fascia, resulting in additional damage to the injured muscle. It needs to be emphasized here that sutures might not always provide the required strength to reappose all ruptured muscle fibers, and accordingly, the formation of empty gaps between the ruptured muscle stumps cannot always be completely prevented.

As a general rule of thumb, the surgical repair of the injured skeletal muscle is usually easier if the injury has taken place close to the MTJ, rather than in the middle of the muscle belly, because the fascia overlying the muscle is stronger at the proximity of the MTJ, enabling more exact anatomical reconstruction. In treating muscle injuries with 2 or more overlying compartments, such as the m. quadriceps femoris, one should attempt to repair the fascias of the different compartments separately, beginning with the deep fascia and then finishing with the repair of the superficial fascia.

After surgical repair, the operated skeletal muscle should be supported with an elastic bandage wrapped around the extremity to provide some compression (relative immobility, no complete immobilization, eg, in cast, is needed). Despite the fact that experimental studies suggest that immobilization in the lengthened position substantially reduces the atrophy of the myofibers and the deposition of connective tissue within the skeletal muscle in comparison to immobilization in the shortened position,[70,76,81] the lengthened position has an obvious drawback of placing the antagonist muscles in the shortened position and, thus, subjecting them to the deleterious effects of immobility. After a careful consideration of all the above-noted information, we have adopted the following postoperative treatment regimen for operated muscle injuries: the operated muscle is immobilized in a neutral position with an orthosis that prevents one from loading the injured extremity. The duration of immobilization naturally depends on the severity of the trauma, but patients with a complete rupture of the m. quadriceps femoris or gastrocnemius are instructed not to bear any weight for 4 weeks, although one is allowed to cautiously stretch the operated muscle within the limits of pain at 2 weeks postoperatively. Four weeks after operation, bearing weight and mobilization of the extremity are gradually initiated until approximately 6 weeks after the surgery, after which there is no need to restrict the weightbearing at all.

Experimental studies have suggested that in the most severe muscle injury cases, operative treatment may provide benefits.[6,107] If the gap between the ruptured stumps is exceptionally long, the denervated part of the muscle may become permanently denervated and atrophied.[85] Under such circumstances, the chance for the reinnervation of the denervated stump is improved,[6,85] and the development of large scar tissue within the muscle tissue can possibly be at least partly prevented by bringing the retracted muscle stumps closer together through (micro) surgical means.[1,6,107] However, in the context of experimental studies, it should be noted that the suturation of the fascia does not prevent contraction of the ruptured muscle fibers or subsequent formation of large hematoma in the deep parts of the muscle belly.

RETURN TO SPORT-SPECIFIC TRAINING

The decision regarding the appropriate timing of the return to sport-specific training can be based on 2 simple and inexpensive measures: (1) the ability to stretch the injured muscle as much as the healthy contralateral muscle and (2) the pain-free use of the injured muscle in basic movements. When the patient indicates that she or he has reached this point in recovery, the permission to gradually start sport-specific training is granted.[68,92] However, it should always be emphasized that the final phase of the rehabilitation, the sport-specific training, should preferably begin under the supervision of a coach or a trainer.

THERAPEUTIC ALTERNATIVES

Medication

Similar to many of the issues regarding the most appropriate treatment of muscle traumas, there are few controlled studies on the use of nonsteroidal anti-inflammatory drugs (NSAIDs) or glucocorticoids in the treatment of muscle injuries in humans. However, one study exists on the use of NSAIDs in the treatment of in situ necrosis, the less severe type of muscle injury, which suggested that short-term use of NSAIDs results in a transient improvement in the recovery from exercised-induced muscle injury.[118] Despite the lack of direct human evidence, the effects of NSAIDs have been quite well documented experimentally.[4,5,72,117,152] Short-term use of different NSAIDs in the early phase of healing has been shown to lead to a decrease in the inflammatory cell reaction,[72] with no adverse effects on the healing process or on the tensile strength or ability of the injured muscle to contract.[72,117] Furthermore, the NSAIDs do not hinder the abilities of the activated satellite cells to proliferate or the myotubes to form in situ.[152] However, it seems that the use of NSAIDs should be restricted only to the early phases of muscle repair, as their long-term use seems to be detrimental to the regenerating skeletal muscle, at least in the eccentric contraction-induced strain injury model.[109]

Although the short-term use of NSAIDs started immediately after the injury can be considered a relatively well-justified treatment of muscle injuries without an apparent risk of delaying the healing,[4,5,72,109,117,118,152] the situation seems to be completely opposite concerning the use of glucocorticoids.[12,72] Delayed elimination of the hematoma and the necrotic tissue, retardation of the muscle regeneration process, and ultimately, reduced biomechanical strength of the injured muscle have been reported with the use of glucocorticoids in the treatment of muscle injuries.[12,72]

Therapeutic Ultrasound

Therapeutic ultrasound is widely recommended and is used in the treatment of muscle injuries, although the scientific evidence on its effectiveness is somewhat vague.[127,164] In addition to the fact that the micromassage produced by high-frequency ultrasound waves apparently works as a pain relief, it has been proposed that ultrasound could somehow enhance the initial stage of muscle regeneration. However, despite the apparent promotion of the proliferation phase of the myoregeneration,[127] therapeutic ultrasound unfortunately does not seem to have a positive (muscle-healing enhancing) effect on the final outcome of muscle healing.[127,164]

Hyperbaric Oxygen Therapy

Hyperbaric oxygen therapy has been proposed as another intriguing therapeutic option to improve the regeneration of the injured skeletal muscle.[14] As described in more detail previously, the restitution of the blood supply to the injured area is the first sign of the initiation of the regeneration process and an apparent prerequisite for the successful continuation of the later stages of the regeneration process, as the multinucleated young myotubes depend solely on aerobic metabolism as the source of energy required for their regeneration.[75] This obvious dependence of the recovery of the injured muscle on the adequate restoration of the vasculature/supply of oxygen[68,75,82] also provides a solid theoretical basis for the use of hyperbaric oxygen therapy in the treatment of injured skeletal muscle.

A recent experimental study actually showed that the use of hyperbaric oxygen therapy applied during the early phase of the repair considerably accelerated the recovery of the injured skeletal muscle.[14] However, both the authors themselves and the research committee of the AOSSM commenting on the study emphasized that caution should be exercised in extending these experimental findings to clinical practice, as there are no clinical studies to show the beneficial effects of this therapy in the treatment of muscle or other types of soft tissue injuries in athletes.[14,128]

COMPLICATIONS: MYOSITIS OSSIFICANS TRAUMATICA

Myositis ossificans is a non-neoplastic proliferation of bone and cartilage within the skeletal muscle at the site of a previous single major trauma or repeated injury and/or hematoma. Being a relatively rare complication of muscle injury, the scientifically valid evidence regarding either the pathogenesis or the most optimal treatment is virtually nonexistent.[11] In sports, myositis ossificans is typically associated with prior sports-related muscle injury, with the incidence being the highest in the high-contact sports in which the use of protective devices is uncommon (eg, rugby).[11] Increased susceptibility to myositis ossificans has also been described in individuals with hemophilia or other bleeding disorder in conjunction with a soft tissue injury.[11]

Clinically, myositis ossificans should be suspected if pain and swelling have not clearly subsided 10 to 14 days after an injury to a skeletal muscle or if the healing does not seem to progress normally despite the execution of proper nonoperative treatment. One should be particularly

alert if the symptoms intensify weeks after the trauma, especially if the injured muscle area becomes more indurated and the injured extremity displays reduced joint range of motion.[11] Although it is sometimes possible to detect the first signs of the ectopic bone on radiographs as early as 18 to 21 days after the injury, the formation of ectopic bone usually lags behind the symptoms by weeks, and thus, a definite diagnosis based on radiographs can be made substantially later.[11]

Because of its rarity, the treatment principles of myositis ossificans are based more on empirical experience rather than on the clinical or even experimental evidence of any other type of muscle complaint.[9] The proper first aid of muscle trauma (the prevention of the formation of a large hematoma) naturally creates the foundation for the treatment of this complaint. However, if the myositis ossificans still occurs despite the best prevention efforts, there is little that can or should be done in the acute phase. Although indomethacin is quite commonly used in orthopaedics in preventing heterotopic ossification, it has not been validated for the prevention and/or treatment of myositis ossificans.[9] The surgical excision of the ectopic bone mass can be considered at later phases if the symptoms do not recede despite 12 months of watchful waiting. However, according to our experience, surgery should not be performed until the ectopic bone has fully "matured," which is 12 to 24 months after the start of the symptoms, as the excision of immature bone often results in local recurrence. Overall, the myositis ossificans could be considered to underscore the importance of proper initial treatment of athletes with muscle injury: despite the fact that a great majority of muscle injuries heal virtually irrespective of the primary treatment, compromised healing of muscle injury (myositis ossificans) results in a delay in the return to sports that is highly comparable to—and often even longer than—that associated with the failed treatment of other major sports-related injuries.[11]

FUTURE PERSPECTIVE

The therapeutic use of growth factors and gene therapy, alone or in combination, and the application of stem cell–based therapies provide the latest, most promising, but currently also the most poorly validated therapeutic options for the enhancement of the healing of an injured skeletal muscle.

Growth Factors

The growth factors and the cytokines are potent mitogenic activators for numerous different cells including the mpc, and they have been shown to be involved in the activation of mpc during the regeneration of the injured muscle cells.[16,23] Thus, they are naturally a potential therapeutic option for accelerating the recovery of the injured skeletal muscle from injury. However, when considering their therapeutic application, it should be noted that in addition to the above-noted potential therapeutic use of the growth factors, these substances also pose considerable undesired side effects. In brief, despite the obvious stimulatory effects on the proliferation of the myoblasts, growth factors such as TGF-β, HGF, and FGF have been shown to inhibit the differentiation of the myoblasts and the progression of muscle fiber regeneration if expressed (administered) longer than needed.[23,110] Furthermore, the stimulatory effect of growth factors is not specific to muscle cells only, as their influence is exerted on the fibroblasts too.[59] For example, TGF-β is considered to be the growth factor primarily responsible for the scar formation in the wound repair,[59,101] and it can even induce undesired differentiation of myogenic cells into myofibroplastic cells.[101] Thus, there are a number of different experimental strategies being tested to actually inhibit the activity of TGF-β in the injured skeletal muscle.[42,44,59,121]

Considering that in the injured skeletal muscle, the regeneration of the myofibers and the formation of the connective tissue scar are concomitantly both supportive and competitive processes,[62,73,83] the dual stimulatory effect of growth factors could lead not only to accelerated muscle regeneration but also to the formation of excessive scar tissue at the injury site.[59] Furthermore, the activity of the growth factors is controlled by a large number of ECM proteins, namely the heparin sulfate proteoglycans and the SLRPs.[26,27,34,160,166] The binding to the heparan sulfate proteoglycans is an absolute requirement for several growth factors to have any biological activity at all, whereas the SLPRs, in turn, are key modulators for the activity of the growth factors. Thus, it is not surprising that these molecules are also involved in the healing process of the injured skeletal muscle.[26,27,34] Accordingly, because the optimal regeneration of injured skeletal muscle appears to be controlled by a closely regulated expression of growth factors (and/or cytokines) and specific ECM molecules that the growth factors bind and that, in turn, regulate their activity to ensure that the growth factors exert their actions precisely at specific sites and times during the regeneration process,[25,34,160] the therapeutic use of growth factors cannot be seriously considered until a perfect spatiotemporal administration can be achieved.

The feasibility of the direct administration of growth factors to the healing muscle injury has actually been tested experimentally.[91,106,110,145] In their extensive study with 3 different models of muscle injury, Mitchell et al[110] did not see any beneficial effects of the administration of FGF-2 to the injury site. In addition, the overexpression of the skeletal muscle–specific isoform of IGF-1 (ie, *m*IGF-1) did not result in enhanced regeneration in the whole graft model of skeletal muscle regeneration.[136] However, somewhat contradictory to these negative findings, it has also been reported that FGF-2 acts as a powerful stimulus for skeletal muscle regeneration.[91,106] In addition to FGF-2, IGF-1 and, to a lesser extent, nerve growth factors were shown to enhance muscle regeneration; the injured muscles treated with these growth factors showed improved healing and increased resistance to tensile loading in comparison to untreated muscles.[91,106,145] Furthermore, the combination of IGF-I with a specific inhibitor of fibrous scar formation (TGF-β antagonist decorin) has been shown to be very successful, managing to simultaneously stimulate muscle

regeneration and prevent the formation of a fibrous scar.[134]

Gene Therapy

Growth factors and many other genes with potentially favorable effects on muscle healing can theoretically be delivered to the injury site through gene therapy.[42,89,95,145] The basic principle of gene therapy is straightforward. The gene of interest with a desired effect on the biological process in question (here, muscle healing) is first transported (delivered) into the desired cell, currently either directly into the liposomes (naked DNA) or inside viruses that infect the cells.[42,89,95] In gene therapy with viruses, the gene of interest is cloned into vectors that are then transfected into the viruses, and these viruses (carrying the vectors encoding the gene) then infect the cells at the injury site, thus conveying the gene into the target cells. After being delivered into the target cell, the gene starts to encode the desired gene product and, thus, should produce the desired (beneficial) biological effect(s) on the target tissue with perfect site specificity.[42,89,95]

Injured skeletal muscle provides an optimal model for testing the potential of gene therapy, as the large number of activated myoblasts within each regenerating myofiber provides a large enough pool of potential targets for the transferred gene to bring about an effective gene expression and the resulting desired biological effect. However, only the very first steps have been taken on this path, and future studies will eventually demonstrate whether gene therapy can uphold the current high expectations as a potentially effective means to treat patients with skeletal muscle trauma.

Stem Cells

Stem cells are undifferentiated cells capable of proliferation, self-renewal, production of a large number of differentiated progeny, and regeneration of tissues.[29,121] Postnatal stem cells can be divided into 2 categories: the tissue-nonspecific and tissue-specific stem cells. The former are hematopoietic in origin and can differentiate into different blood lineages,[121] whereas the latter preferentially differentiate into cells of the residing tissue, although they also possess a limited ability to transform into other lineages.[121,123,137]

Regarding the stem cells and muscle healing, it was recently shown that in response to injury, not only the tissue-specific stem cells (classical and extralaminal satellite cells) but also the nonmuscle resident stem cells participate in the repair process. The latter seem to invade the site of injury, differentiate into satellite cells, and participate in the repair of the injured skeletal muscle.[29,46,94] As described previously, it seems that there are at least 3 different populations of satellite cells in the skeletal muscle.[‡‡] In terms of their therapeutic potential, unmodified muscle-derived stem cells have been proven capable of regeneration in muscular dystrophies.[121] Furthermore, when genetically modified to express growth factors, these cells are versatile in promoting healing of the injuries in different musculoskeletal tissues, such as in bone (fractures) and in ligaments (ruptures), or in inhibiting undesired effects, such as heterotrophic bone formation.[54,114,120,169] On the other hand, part of the biological activity for some of the growth factors, such as *m*IGF-1, is derived from their ability to recruit BM-derived stem cells efficiently to the site of the damage.[113] Thus, stem cell–based therapy, particularly genetically engineered therapy, holds great potential for the treatment of a variety of disorders and conditions affecting the injured skeletal muscle and its connective tissue.[121]

[‡‡]References 40, 66, 88, 123, 125, 130, 137.

SUMMARY

Only a few clinical studies exist on the treatment of muscle injuries, and thus, the current treatment principles of muscle injuries are mostly based on experimental studies or empirical evidence only. Experimental studies have shown that the basic biological processes occurring in the healing muscle are identical, irrespective of the primary cause of the injury (contusion, strain, or laceration). This finding naturally places the importance of understanding the basic principles of muscle healing into sharpened focus, as they form the basis for the proper execution of treatment of muscle injury.

Clinically, the first aid of muscle injuries follows the RICE principle, the principle common to the treatment of any soft tissue trauma. The objective of the use of the RICE principle is to stop intramuscular bleeding and thereby limit the progression of the muscle injury to a minimum. Clinical examination should be carried out immediately after the trauma and 5 to 7 days thereafter, at which point the imaging modalities (MRI or ultrasound) can provide useful insights into the severity of the injury. During the first few days after the injury, a short period of immobilization accelerates the formation of granulation tissue at the site of injury, but it should be noted that the duration of reduced activity (immobilization) ought to be limited only until the scar reaches sufficient strength to bear the muscle contraction–induced pulling forces without rerupture. At this point, gradual mobilization should be started to optimize the healing by restoring the strength of the injured muscle and preventing muscle atrophy, loss of strength, and extensibility, all of which can follow prolonged immobilization.

Despite the common belief that injured skeletal muscle undergoes fibrosis during repair, the amount of intramuscular connective tissue is not increased in the injured skeletal muscle unless the injured limb is immobilized for a prolonged period of time. Furthermore, although extensive granulation tissue formation takes place after an injury to the skeletal muscle, the wound contracts very efficiently into a small fibrous scar without any need for pharmacological or surgical interventions.

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