CURRENT CONCEPTS REVIEW
MUSCLE INJURIES AND REPAIR: CURRENT TRENDS IN RESEARCH

BY JOHNNY HUARD, PHD, YONG LI, PHD, MD, AND FREDDIE H. FU, MD

Investigation performed at the Growth and Development Laboratory, Children's Hospital of Pittsburgh; the University of Pittsburgh; and the Department of Orthopaedic Surgery, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

- After injury, muscle healing occurs through different phases, including (1) degeneration and inflammation, (2) muscle regeneration, and (3) development of fibrosis.
- The severity and type of muscle injury influence the healing process.
- Enhancement of muscle regeneration and prevention of muscle fibrosis can improve muscle healing.
- Growth factors, including basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF-1), and nerve growth factor (NGF), can improve muscle regeneration, but the post-injury healing process remains incomplete.
- The use of anti-fibrosis agents that antagonize the effect of transforming growth factor-β1 (TGF-β1) can prevent fibrosis and improve muscle healing, resulting in nearly complete recovery.
- Optimal muscle recovery may require the use of novel technologies, such as gene therapy and tissue engineering, to achieve both high levels and long-term persistence of these growth factors and cytokines within the injured muscle.

Muscle injury presents a challenging problem in traumatology, as injured muscles heal very slowly and often with incomplete functional recovery. It has been observed that injured muscles can initiate regeneration promptly, but the healing process is often inefficient and hindered by the formation of scar tissue, which may contribute to the tendency for muscle injury to recur. The enhancement of muscle regeneration and the prevention of muscle fibrosis through the use of biological approaches are being investigated in an effort to improve muscle healing after injury. In this Current Concepts Review, we will outline the structure and histological organization of skeletal muscle and describe the basic physiology of skeletal muscle contraction. We will subsequently summarize the biological and pathological processes that occur in skeletal muscle after injury (i.e., degeneration, inflammation, regeneration, and fibrosis) and present the clinical treatments currently available for injured skeletal muscle. Finally, we will discuss current trends in research, which include the improvement of regeneration and the inhibition of fibrosis in injured skeletal muscle. By enhancing our understanding of the muscle healing process, it may be possible to develop more effective biological approaches to improve muscle healing and obtain complete functional recovery.

Skeletal Muscle: Structure and Function

Skeletal muscle represents the largest tissue mass in the body, constituting 40% to 45% of total body weight. It is a composite structure consisting of muscle cells, organized networks of nerves and blood vessels, and an extracellular connective-tissue matrix[1]. This framework is necessary both to produce joint movement and locomotion and to support the regeneration process that occurs after injury. The basic structural element of skeletal muscle is the muscle fiber or myofiber (Fig. 1). The cytoplasm of the myofiber, called the *sarcoplasm,* contains a cellular matrix and organelles, including the Golgi apparatus, mitochondria, sarcoplasmic reticulum, lipid droplets, glycogen, and myoglobin. The skeletal muscle fiber is a syncytium derived from the fusion of multiple myoblasts (myogenic precursor cells). Briefly, the myoblasts fuse to form long, cylindrical, multinucleated myotubes that exhibit central nucleation. Once the myonuclei shift from a central position to a subsarcolemmal position, the muscle cells are usually termed *myofibers* (Fig. 1). In fact, the appearance of central nuclei within otherwise normal adult muscle can be an indication of muscle regeneration under certain conditions, as described.

The endomysium is the connective-tissue layer that surrounds individual myofibers, whereas the perimysium sur-

rounds fascicles or bundles of myofibers. The epimysium is the outside connective-tissue layer that surrounds the skeletal muscle (Fig. 1). The fiber arrangement, which is an important determinant of the functional and contractile properties of the skeletal muscle, may be parallel or oblique to the long axis of the muscle.

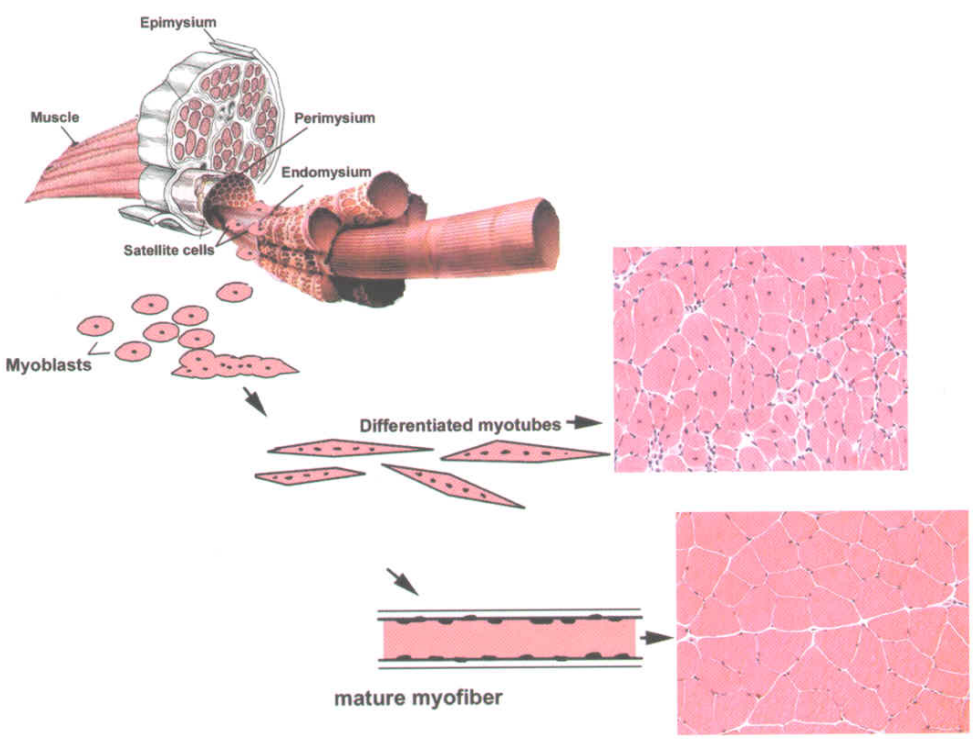
The sarcolemma is the plasma membrane that surrounds each myofiber unit. The basal lamina or basement membrane, which constitutes the 100 to 200-nm-thick external connective-tissue layer, is composed of an inner layer, an intermediate lucida, and the outer lamina densa[1]. The basement membrane contains a number of proteins, including collagen, fibronectin, laminin, and many glycoproteins. Each myofiber contains a multitude of nuclei derived from myoblasts located at the periphery of the myofibers. In addition, separate cells called *satellite cells* are located between the basal lamina and plasma membrane and play a key role in the muscle regeneration process[2,3]. It has become clear from the work of many investigators over the past three years that satellite cells proliferate following muscle trauma and form new myofibers through a process equivalent to muscle histogenesis in the embryo. Upon activation after focal injury, the degeneration of one or more myofibers results in satellite cell proliferation. This process is limited to areas where there is necrosis of myofibers. Satellite cell progeny (myoblasts) begin to fuse and form multinucleated myotubes after a few cell divisions, but the proliferation of satellite cells can continue for nine to ten days, depending on the severity of the injury (Fig. 1).

Skeletal muscle function is under the control of a nerve that enters the muscle at its motor point. Each nerve-cell axon branches many times, and each myofiber is contacted by one nerve terminal (Fig. 2). The point of contact between the myofiber and the nerve is called the *motor end plate of the neuromuscular junction*. The neuromuscular junction contains three major structural components: the presynaptic axon, the synaptic cleft, and the postsynaptic area on the myofibers. The single nerve axon and all of the myofibers that it contacts constitute a motor unit. Both the number of myofibers within a motor unit and the number of motor units per skeletal muscle vary according to the type of movement made by a given muscle. In fact, where fine motor control and highly coordinated movement are necessary, such as in the extraocular muscles, there may be as few as ten myofibers per motor unit. This contrasts with large skeletal muscles, such as the gastrocnemius, which contain up to 1000 myofibers per single motor unit. In general, the initial step in muscle contraction involves the release of acetylcholine by the presynaptic axons in the synaptic clefts. The acetylcholine released in the synaptic cleft binds to the acetylcholine receptors of the postjunctional folds of the myofibers (postsynaptic area) and consequently depolarizes the cell (Fig. 2). The depolarization triggers an action potential that passes along the length of the myofibers, resulting in muscle contraction. After the depolarization of the end plate,

cell divisions, but the proliferation of satellite cells can con- muscle contraction. After the depolarization of the end plate,

Fig. 1

Schematic drawing of the structural design of skeletal muscle. The endomysium is the connective-tissue layer that surrounds individual myofibers, the perimysium surrounds fascicles or bundles of myofibers, and the epimysium is the outside connective-tissue layer that surrounds the skeletal muscle. On muscle injury, the satellite cells are released and are activated to become myoblasts, which eventually differentiate into immature (myotubes) and mature muscle fibers. The nuclei are located in the central portion of the myotubes (immature myofibers), but they eventually migrate to the periphery of the myofiber when the muscle fibers mature.



the electric impulse passes along the muscle membrane to reach the interior of the muscle by means of the transverse tubule. This causes a momentary release of calcium from the sarcoplasmic reticulum[1]. The calcium that is released causes the contractile proteins (actin and myosin) to interact and to generate force in a stepwise manner. The calcium is first released from the sarcoplasmic reticulum and binds to troponin, which is a component of the actin filament to which myosin binds. The calcium subsequently causes a conformational change of the troponin. This allows the interaction between actin and myosin to occur and consequently results in muscle contraction. Finally, at the end of muscle contraction, the acetylcholine is deactivated by the enzyme acetylcholinesterase (or by other less specific cholinesterases), muscle relaxation occurs, the intracellular calcium is transported in the transverse tubules within the myofiber, and the troponin prevents the interaction between the actin and myosin molecules[1].

Different types of muscle contraction can occur (Fig. 3). In isometric contraction, the force generated by the muscle is equal to the resisting load and, therefore, the length of the muscle does not change. In concentric contraction, the force generated by the muscle is larger than the resisting load and causes the muscle to shorten. Isometric contraction can be voluntarily produced—for example, by lifting a weight and maintaining the forearm in a partially flexed position. The amount of muscle tension produced can then be increased by recruiting more muscle fibers until the muscle begins to shorten; at this point, isometric contraction is converted to concentric contraction. In eccentric contraction, the resisting load is larger than the force generated by the skeletal muscle and causes the muscle to lengthen.

Conditions of nerve activation can be controlled to produce a single stimulus, repetitive stimuli at constant frequencies, or stimuli in other desired modes. When a skeletal muscle is stimulated with a single electric shock of a sufficient voltage, it quickly contracts and then relaxes. This response is called *single twitch*. If the stimulator is set to deliver an increasing frequency of electric shocks automatically, the relaxation time between successive twitches will get shorter and shorter as the strength of contraction increases in amplitude. This response

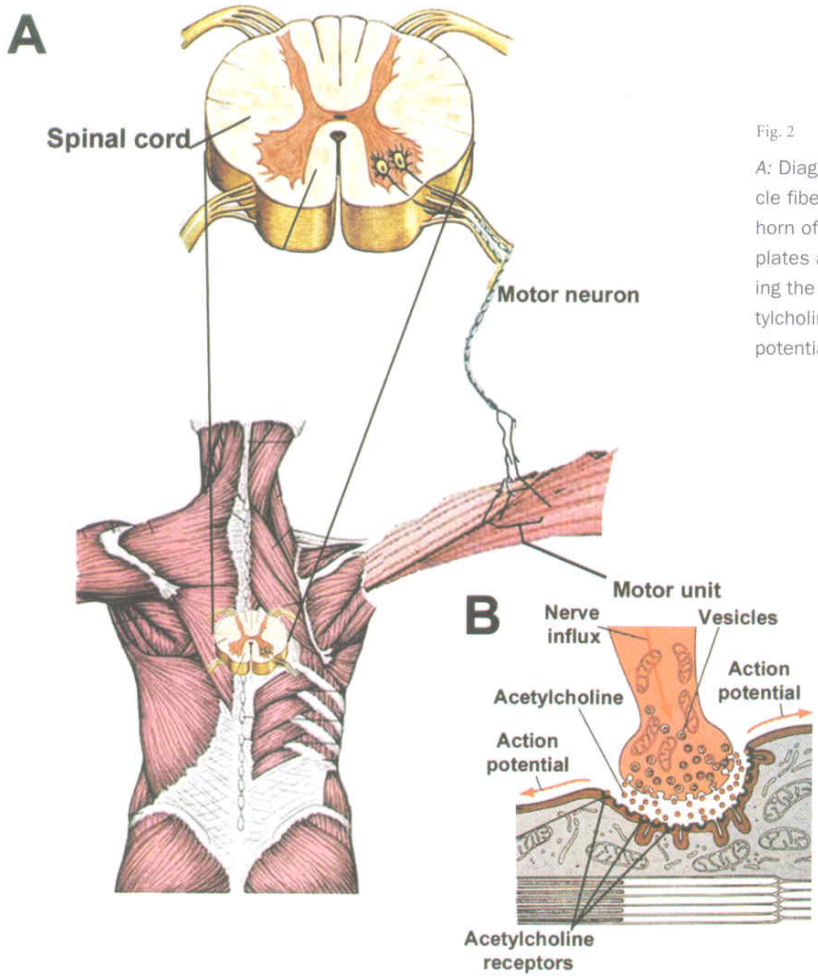


Fig. 2

A: Diagram illustrating the innervation of a muscle fiber by a motor neuron located in the ventral horn of the spinal cord. *B:* Diagram of motor end plates at the neuromuscular junctions, illustrating the direction of the nerve influx, vesicles, acetylcholine, acetylcholine receptors, and action potential migrating along the muscle fibers.

is called a *paired twitch* or *incomplete tetanus*. Finally, at a particular "fusion frequency" of stimulation there is no visible relaxation between successive twitches; this leads to a sustained muscle contraction called *tetanus*[1].

In order to move a joint, individual skeletal muscles work as a group. The first muscles involved in a particular movement are called *agonists*. Muscles that provide resistance, generally on the opposite side of a joint, are called *antagonists*. For example, when the elbow is brought into flexion the anterior muscles (such as the biceps) of the arm are agonists while the posterior muscles (such as the triceps) are antagonists. However, when the elbow moves into extension the anterior muscles become antagonists while the posterior muscles are agonists.

Several muscles may also be involved in a specific movement by immobilizing a joint and acting as force couples that prevent undesirable movement. Moreover, the composition of the skeletal muscle may change in relation to the type of exercise and performance required. There are three major types of myofibers: type 1, which are slow myofibers that are resistant to fatigue; type 2A, which are fast myofibers that are resistant to fatigue at an intermediate level between the levels of types 1 and 2B; and type 2B, which are also fast myofibers but are not resistant to fatigue[1]. Although most human skeletal muscles are composed of a mixture of muscle fiber types, the type of performance may change the overall distribution of muscle fiber types in a given muscle[4]. For example, muscle biopsies performed on elite sprinters are more likely to show a larger number of type-2 myofibers, whereas biopsies performed on distance runners are more likely to show an increased number of type-1 myofibers[4]. In general, the force produced by a skeletal muscle is proportional to its physiological cross-sectional area, but the total amount and speed of shortening are proportional to the individual muscle fiber's length and type[1].

Biological Process of Skeletal Muscle Healing Following Injury

Muscle injuries occur through a variety of mechanisms, including direct trauma (e.g., lacerations, contusions, and strains) and indirect causes (e.g., ischemia and neurological dysfunction)[5-19]. The different phases of healing occurring within the damaged muscle are similar among the various types of muscle injuries, but the functional recovery of the injured muscle varies from one type of muscle injury to another. After many years of research, it has become clear that the processes occurring in injured muscle (i.e., necrosis/degeneration, inflammation, repair, and scar-tissue formation [fibrosis]) are all interrelated and time-dependent[5-19].

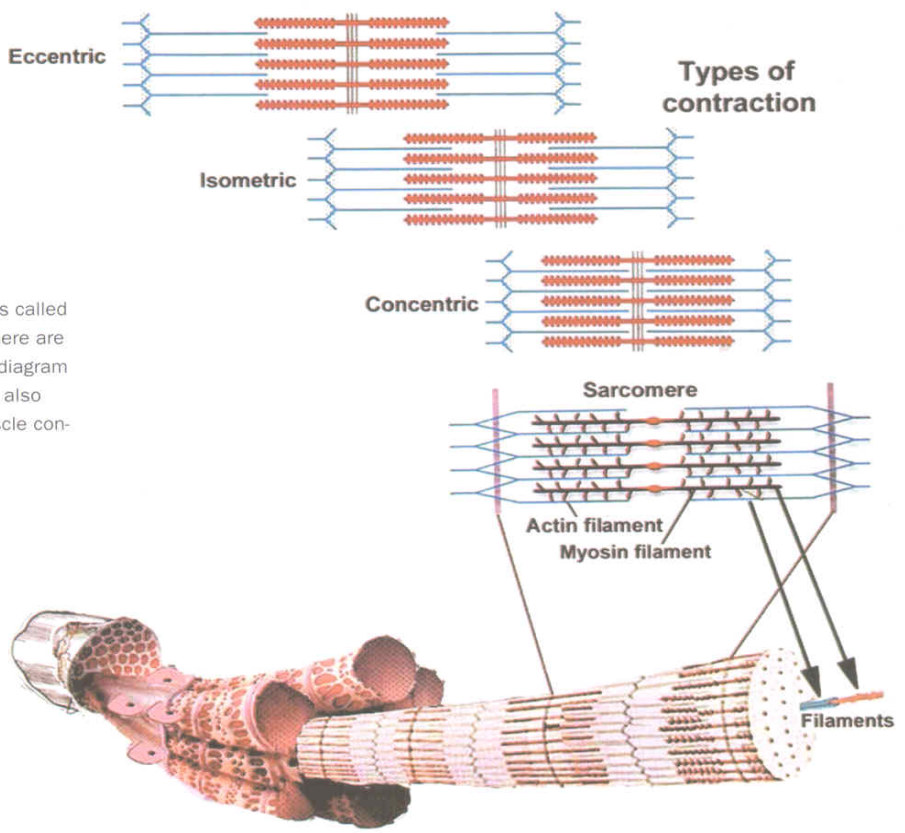


Fig. 3

The myofibrils, the major constituent of the muscle fibers, are made up of contractile units called *sarcomeres*. The key components of a sarcomere are two filamentary proteins, actin and myosin. A diagram of the sliding filament model of contraction is also presented for the three different types of muscle contraction.

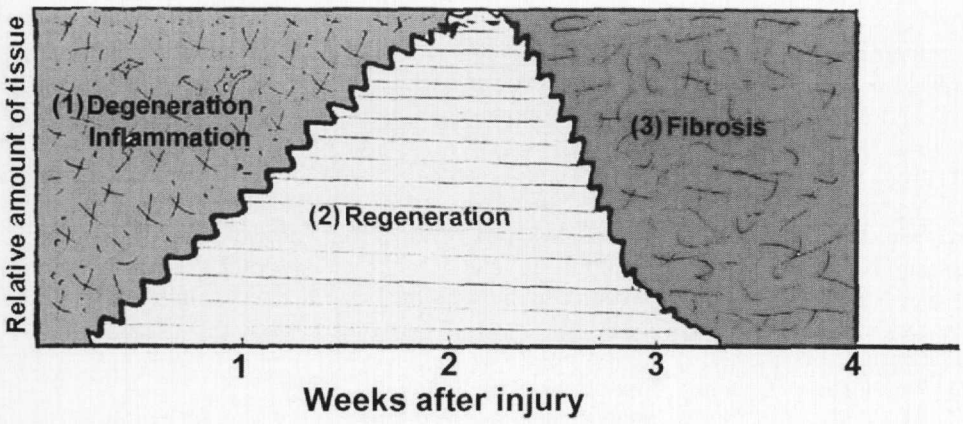


Fig. 4
The different stages of muscle healing after muscle injury. The first event is muscle degeneration and inflammation, which occurs within the first minutes and continues for up to one to two weeks after injury. Muscle regeneration begins in the first week post-injury and peaks at about fourteen days post-injury. Fibrosis usually occurs at two weeks post-injury and increases over time for up to four weeks post-injury.

In injured muscle, mechanical trauma destroys the integrity of the myofiber plasma membrane and basal lamina, leading to the ingress of extracellular calcium[11,19,20]. The injured myofibers undergo necrosis by autodigestion mediated by intrinsic proteases[21,22]. Local swelling and hematoma formation occur rapidly after injury and further promote muscle degeneration[11,23,24]. Subsequently, the necrotic area is invaded by small blood vessels, and mononuclear cells, activated macrophages, and T-lymphocytes infiltrate the local tissue. These activated lymphocytes simultaneously secrete several cytokines and growth factors, which perform a wide range of functions in the inflammation process[24-26]. The secretion of substances such as adhesion molecules (e.g., P-selectin, L-selectin, and E-selectin) and cytokines (e.g., interleukins [IL-8, IL-6, IL-1] and tumor necrosis factor-α [TNF-α]) influences local blood flow and vascular permeability and accelerates the inflammatory response[24-30]. More importantly, the release of growth factors such as insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factors (TGF-α and TGF-β), and platelet-derived growth factors (PDGF-AA and PDGF-BB) at the injured site also regulates myoblast proliferation and differentiation to promote muscle regeneration and repair (Table I). This phase is associated with the activation of satellite cells as described above[31-33]. It is hypothesized that, after muscle injury, disruption of the basal lamina and plasma membrane releases and activates the satellite cells[1-3]. The satellite cells, under the influence of various growth factors, then become activated, proliferate, and differentiate into multinucleated myotubes and eventually into regenerated myofibers.

After injury, muscle undergoes a distinct set of healing phases, consisting of degeneration, inflammation, regeneration, and fibrosis (Fig. 4). Active muscle degeneration and inflammation occur in the first few days post-injury, whereas muscle regeneration usually occurs seven to ten days after injury. The regeneration process usually peaks at two weeks and then decreases at three to four weeks post-injury. The formation of scar tissue (fibrosis) begins between the second and third weeks postinjury, and the scar tissue increases in size over time. The formation of scar appears to be the end product of the muscle repair process. We therefore believe that as long as scar is formed, complete regeneration of muscle tissue cannot occur. On the basis of the biological process of muscle healing described above, the development of biological approaches to enhance muscle regeneration and prevent muscle fibrosis to improve the potential for muscle healing post-injury is being investigated.

Current Trends in Research

Improving Muscle Regeneration

Use of human recombinant growth factors to improve muscle healing

Many growth factors stimulate growth and protein secretion in various musculoskeletal cells[34]. Preliminary data suggest that growth factors play a variety of roles during muscle regeneration[34-58]. Insulin-like growth factor-1 (IGF-1) is of particular interest since it is highly mitogenic for myoblasts[59-62]. Although other growth factors, including fibroblast growth factors (FGF) and platelet-derived growth factors (PDGF), display potent stimulating effects on satellite cell proliferation[35,40,58], IGF-1 appears to be critical in mediating the growth of skeletal muscle[63]. Systemic administration of IGF-1 results in increased muscle protein content and reduced protein degradation[64]. More importantly, transgenic mice overexpressing human IGF-1 exhibit muscle hypertrophy[65]. In a study of healthy older men, the loss of muscle mass was prevented when endogenous levels of IGF-1 were induced by exogenous administration of growth hormone[66]. Recently, gene transfer of IGF-1 by an adeno-associated viral (AAV) vector into mouse skeletal muscle was found to block the well-documented age-related loss of muscle mass and function[67,68]. However, IGF-1, a potent mitogen for fibroblasts, can increase production of matrix components such as collagen and decrease expression of matrix-degrading enzymes such as collagenase, potentially resulting in the development of fibrosis following muscle injury[69].

In a mouse model, IGF-1, basic fibroblast growth factor (bFGF), and to a lesser extent nerve growth factor (NGF) directly injected at two, five, and seven days post-injury were shown to enhance regeneration in lacerated, contused, and strain-injured muscle[11-13,16]. The number of regenerating myofibers substantially increased seven days after injection. The diameter of the regenerating myofibers in the treated muscle

TABLE I Effect of Growth Factors on Myoblasts in Vitro

Growth Factor	Cell Proliferation	Cell Differentiation
Hepatocyte growth factor (HGF)	*Stimulates*	*Stimulates*
Basic fibroblast growth factor (bFGF)	Stimulates	Stimulates
Insulin-like growth factor-1 (IGF-1)	Stimulates	Stimulates
Nerve growth factor (NGF)	Stimulates	Stimulates
Leukemia inhibitory factor (LIF)	Stimulates	Stimulates
Acid fibroblast growth factor (aFGF)	*Inhibits*	*Stimulates*
Platelet-derived growth factor (PDGF-AA)	Inhibits	Stimulates
Platelet-derived growth factor (PDGF-BB)	Stimulates	Inhibits
Epidermal growth factor (EGF)	Inhibits	Inhibits
Transforming growth factor-α (TGF-α)	Inhibits	Inhibits
Transforming growth factor-β1 (TGF-β1)	*Inhibits*	*Inhibits*

also substantially increased, indicating an acceleration of muscle regeneration in the injured tissue treated with these growth factors. Furthermore, an overall improvement in strength (tetanic and fast twitch strength) was observed in the injured mouse muscle treated with IGF-1, bFGF, and NGF at fifteen days after the injection[11-13,16].

There are additional growth factors that activate satellite cells and enhance muscle regeneration after injury (Table I). Hepatocyte growth factor (HGF) and leukemia inhibitory factor (LIF) are two good examples of satellite cell stimulators[17,38,51,56,70-72]. Although HGF can activate quiescent satellite cells in skeletal muscle, the injection of HGF directly into an injured muscle does not promote repair[17]. Preliminary data indicate that LIF can improve muscle healing, but there is need for additional research investigating this effect[38]. Other factors, such as vascular endothelial growth factor (VEGF), can improve healing of ischemic muscle by stimulating angiogenesis[73,74]. It is clear that the dosages of these growth factors play a major role in their ability to improve muscle healing, as described below.

Gene Therapy to Deliver Growth Factors into Injured Skeletal Muscle

One potential advantage associated with using human recombinant growth factors in the treatment of muscle injuries is the ease and safety of the injection procedure. However, the direct injection of recombinant proteins (growth factors) is limited by the high concentration of the factor typically required to produce a substantial effect. Indeed, it has been shown that growth factors exhibit a dose-dependent effect on myoblast proliferation and differentiation in vitro whereas, in vivo, three consecutive injections of a relatively high concentration of NGF, IGF-1, and bFGF (100 ng of growth factor) are usually required to achieve detectable enhancement of healing of skeletal muscle in mice[11-13,16]. The bloodstream's rapid clearance of the molecules and the molecules' relatively short biological half-lives are the main reasons that large concentrations of growth factor are typically required. Gene therapy may prove to be an effective method for delivering stable high concentrations of growth factor to injured muscle.

Both viral vectors (adenovirus, retrovirus, herpes simplex virus, and adeno-associated virus) and nonviral vectors (plasmid DNA and liposomes) have been used to deliver genes to injured skeletal muscle[75,76]. Each of these vector systems has advantages and disadvantages, but the ability of the adenovirus to efficiently transduce regenerating myofibers[75,76] has prompted researchers to focus on adenoviral vectors as promising gene delivery vehicles. Direct intramuscular or myoblast-mediated ex vivo gene transfer of recombinant adenovirus carrying a so-called *reporter gene* (β-galactosidase) was shown to be highly efficient in the treatment of lacerated, contused, and strain-injured skeletal muscle[11,12,14]. (Reporter genes, which encode for easily detectable non-therapeutic proteins, provide evidence that the transduced cells express the gene properly.) Many β-galactosidase-expressing myofibers were found in the injured sites five days after either direct or ex vivo gene transfer.

Since previous studies have shown that IGF-1 is a potent growth factor in stimulating muscle regeneration and improving muscle healing in vivo following injury[11-13,16], an adenovirus carrying the IGF-1 gene was engineered and was evaluated for its ability to improve muscle healing following injury[14]. In a mouse model, the direct injection of the adenovirus IGF-1 (Ad-IGF-1) vector into lacerated muscles did not significantly improve muscle strength (fast twitch and tetanic strength) at two weeks after injection. While the myoblast-mediated ex vivo gene transfer by adenovirus IGF-1 (myob/Ad-IGF-1) did improve muscle healing after laceration in immunocompetent

mice[14], a better healing process was observed with the transplantation of the same number of myoblasts alone (without Ad-IGF-1). Although an immune response against the adenovirus may limit the success of gene therapy[75,76], the lack of improvement in severe combined immunodeficient (SCID) mice suggests that the immune response is not a major factor in this lack of improvement in muscle healing. Histologically, the SCID mouse muscle, which was lacerated and injected with adenovirus IGF-1 and IGF-1-expressing myoblasts, showed the development of muscle fibrosis within the lacerated site even though a high level of IGF-1 was produced[14]. Taken together, these results suggest that a high level of IGF-1 secretion mediated by adenoviral-based gene therapy can improve muscle healing but the functional recovery of the injured muscle remains impaired. The stimulatory action of IGF-1 on myofibroblast proliferation and the deposition of extracellular matrix may interfere with the ability of this growth factor, even at high concentrations, to improve muscle healing after injury[69].

Another promising technique that may overcome these hurdles has been reported recently. Using a novel DNA controlled-release device, researchers reported efficient and persistent gene delivery of the reporter gene (alkaline phosphatase) to skeletal muscle[77]. Although this new technology looks promising, more research is required to evaluate its utility in specific applications, including muscle repair.

Inhibition of Fibrosis

Use of an Operative Procedure to Prevent Muscle Fibrosis

The effect of surgical repair (suture) compared with that of a short period of immobilization (five days) on the development of muscle fibrosis and the overall healing process of a lacerated skeletal muscle was investigated in our laboratory[15]. Forty-four adult mice were divided into four groups: laceration of the gastrocnemius muscle followed by surgical (suture) repair, laceration of the gastrocnemius muscle followed by immobilization, laceration of the gastrocnemius muscle only (injured control group), and no injury (uninjured control group). The natural course of muscle recovery was monitored with histological and immunohistochemical analyses as well as functional testing at two, seven, ten, fourteen, and twenty-eight days post-injury. Suturing the lacerated muscle immediately after injury promoted healing and prevented the development of a deep scar, although a superficial scar was still observed. In contrast, immobilization resulted in slower muscle regeneration and the development of a large scar within the injured muscle. This single study suggests that immobilization of the lacerated muscle has no major effect on the development of fibrosis, whereas suturing can limit the fibrosis deep in the injured muscle but cannot eliminate superficial fibrosis.

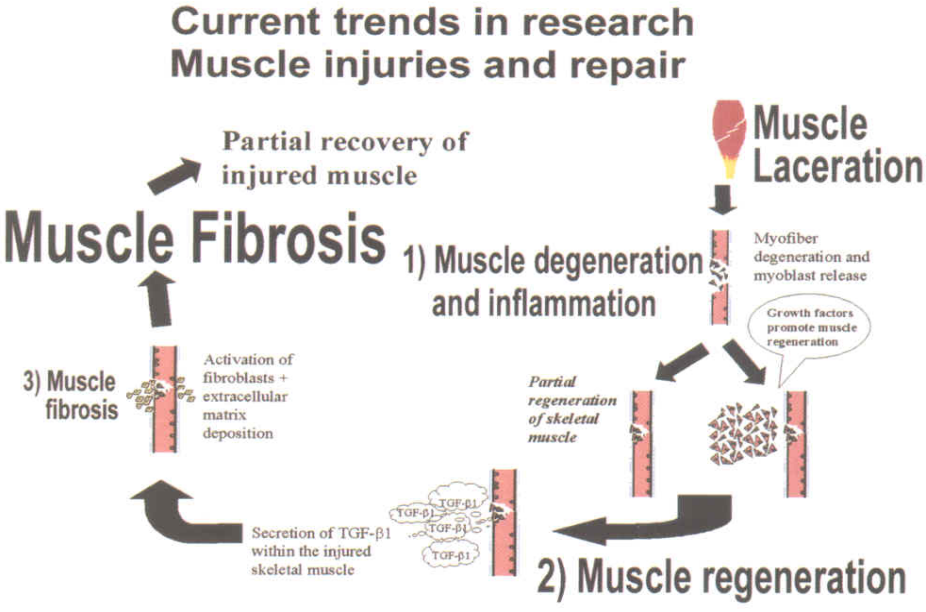


Fig. 5

Improvement of muscle healing post-injury. Muscle injuries induce myofiber degeneration and inflammation in the injured area. Infiltrating lymphocytes release growth factors that activate myoblasts to proliferate and differentiate into myotubes and myofibers at the injured site. The use of growth factor to promote myoblast proliferation and differentiation is a potential way to enhance muscle regeneration after muscle injury. The release of transforming growth factor-β1 (TGF-β1) within the injured site stimulates extracellular matrix deposition and triggers the formation of fibrosis. Blocking the action of TGF-β1 is a potential way to inhibit scar tissue formation and consequently to promote muscle healing post-injury.

Antifibrotic Therapy by Blocking Overexpression of TGF-β1

Although TGF-β1 has been implicated in the development of fibrosis in various tissues[78-96], very few reports have demonstrated the role of this cytokine in skeletal muscle fibrosis. It has been reported, however, that TGF-β1 is expressed at high levels and is associated with fibrosis in the skeletal muscle of patients with Duchenne muscular dystrophy[97,98]. The authors of those reports[97,98] suggested that muscle degeneration occurring secondary to the lack of dystrophin is followed by myofiber necrosis. An inflammatory reaction may take place in the area of necrosis and lead to the focal release of TGF-β1, which triggers fibrosis through extracellular matrix activation and connective-tissue proliferation. An excess of TGF-β1 also has been observed in muscle biopsy specimens from patients with dermatomyositis. This excess TGF-β1 leads to chronic inflammation, fibrosis, and accumulation of extracellular matrix[99,100]. We too have observed (with immunohistochemical analysis) a strong expression of TGF-β1 within diseased and injured skeletal muscle in animal models. These results support the theory that the expression of TGF-β1 in skeletal muscle may play an important role in the fibrotic cascade following the onset of muscle disease or trauma. Therefore, it is conceivable that preventing fibrosis by neutralizing TGF-β1 expression in injured muscle could inhibit the formation of scar tissue. Indeed, the use of anti-fibrosis agents (e.g., decorin) that inactivate this molecule can reduce muscle fibrosis and consequently improve muscle healing, leading to a more nearly complete recovery after laceration[6]. Other candidate agents, such as gamma-interferon and suramin[101-106], are also being investigated for their antifibrotic properties. Gamma-interferon is currently used to treat liver fibrosis and, because it has already been approved by the Food and Drug Administration, is particularly appealing for expedited clinical use[107,108]. Suramin is also clinically available and has been shown to break down collagen after its deposition[105,106]. This product could be extremely useful for the elimination of scar tissue that is already present within healing skeletal muscle.

Because of the apparently critical role of TGF-β1 in the development of tissue fibrosis, this molecule could become a key target for developing strategies to prevent fibrosis in healing skeletal muscle. Various approaches to antagonize the effect of TGF-β1 are currently being investigated; however, blocking the effects of TGF-β1 may not be the only method available for the prevention of fibrosis. Blocking the effects of other molecules involved with the fibrotic cascade, such as collagen deposition, may one day prove to be an effective alternative method. A schematic representation of the different phases of muscle healing after laceration and the beneficial effect of growth factors and anti-fibrosis agents on the healing process is shown in Figure 5. It is important to note that since the muscle repair process may differ depending on the severity and type of injury, approaches to improve muscle healing may also be variable and may require combined therapies. Indeed, the development of approaches to improve healing within an injured muscle in which scar tissue has already developed may require the elimination of the scar tissue prior to the enhancement of muscle regeneration and the prevention of extracellular matrix deposition.

Overview

The best treatment for muscle injuries has not been clearly defined. Consequently, recommended conservative treatment regimens vary widely depending on the severity of the injury. In addition to these conservative treatments, operative treatments can be used, especially for acute injuries such as serious strains that lead to the development of a large intramuscular hematoma. Although all of the above treatments have been used in the clinical setting with good outcomes, the functional recovery of injured skeletal muscle remains limited[5,8,10,14,23]. Consequently, basic research exploring additional ways to improve muscle healing has expanded. The realization that injured skeletal muscle can promptly initiate regeneration but is likely hindered by fibrosis is critical to the development of biological approaches to improve muscle healing post-injury. The delivery of human recombinant proteins to injured skeletal muscle either by direct injection or through the use of gene therapy represents potential methods described in this review for enhancing muscle regeneration and inhibiting fibrosis. Continued research should improve our incomplete understanding of the muscle healing process, expedite the development of methodology necessary to promote efficient muscle healing to achieve complete functional recovery, and perhaps contribute to the development of innovative therapies for congenital muscle diseases. ■

NOTE: The authors thank Marcelle Pellerin for her technical assistance as well as Lauren Rudick, Ryan Sauder, and Jim Cummins for their careful reading and editing of the manuscript. The authors also wish to thank the following people who have been involved in this research project: Channarong Kasemkijwattana, MD; Jacques Menetrey, MD; Chang Woo Lee, MD; Kazu Fukushima, MD; Kenji Sato, MD; Horaguchi Takashi, MD; Yi-Sheng Chan, MD; Charles Day, MD; Patrick Bosch, MD; Douglas Musgrave, MD; Joon Y. Lee, MD; Vonda Wright, MD; Boonsin Buranapanitkit, MD; Morey S. Moreland, MD; George Somogyi, PhD; Simon C. Watkins, PhD; Molly Vogt, PhD; Paul D. Robbins, PhD; Neil Badlani, BSc; William Foster, BSc; Thomas Payne, BSc; Ryan Pruchnic, BS; Felix Riano, MD; Arvydas Usas, MD; Vladimir Martinek, MD; Xin Lin, MD; and Hsain Chung Shen, MD.

Johnny Huard, PhD
Yong Li, PhD, MD
Growth and Development Laboratory, Children's Hospital of Pittsburgh, 4151 Rangos Research Center, 3705 Fifth Avenue, Pittsburgh, PA 15213.
E-mail address for J. Huard: jhuard+@pitt.edu

Freddie H. Fu, MD
Department of Orthopaedic Surgery, University of Pittsburgh Medical Center, Liliane S. Kaufmann Building, 3471 Fifth Avenue, Suite 1010, Pittsburgh, PA 15213

In support of the research or preparation of this manuscript, one of the authors (J.H.) received grants from the National Institutes of Health (NIH 1 RO1 AR47973-01) and the Orris C. Hirtzel and Beatrice Dewey Hirtzel Memorial Foundation. This work also was supported by the William F. and Jean W. Donaldson Chair at Children's Hospital of Pittsburgh. The authors did not receive payments or other benefits or a commitment or agreement to provide such benefits from a commercial entity. No commercial entity paid or directed, or agreed to pay or direct, any benefits to any research fund, foundation, educational institution, or other charitable or nonprofit organization with which the authors are affiliated or associated.

References

1. **Garrett WE Jr, Best TM.** Anatomy, physiology, and mechanics of skeletal muscle. In: Simon SR, editor. *Orthopaedic basic science*. Rosemont, IL: American Academy of Orthopaedic Surgeons; 1994. p 89-125.
2. **Bischoff R.** The satellite cell and muscle regeneration. In: Engel AG, Franzini-Armstrong C, editors. *Myology. Basic and clinical*. 2nd ed. New York: McGraw-Hill; 1994. p 97-118.
3. **Hurme T, Kalimo H.** Activation of myogenic precursor cells after muscle injury. *Med Sci Sports Exerc.* 1992;24:197-205.
4. **Andersen JL, Schjerling P, Saltin B.** Muscle, genes and athletic performance. *Sci Am.* 2000;283:48-55.
5. **Crisco JJ, Jokl P, Heinen GT, Connell MD, Panjabi MM.** A muscle contusion injury model. Biomechanics, physiology, and histology. *Am J Sports Med.* 1994;22:702-10.
6. **Fukushima K, Badlani N, Usas A, Riano F, Fu F, Huard J.** The use of an antifibrosis agent to improve muscle recovery after laceration. *Am J Sports Med.* 2001;29:394-402.
7. **Garrett WE Jr, Seaber AV, Boswick J, Urbaniak JR, Goldner JL.** Recovery of skeletal muscle after laceration and repair. *J Hand Surg [Am].* 1984;9:683-92.
8. **Garrett WE Jr.** Muscle strain injuries: clinical and basic aspects. *Med Sci Sports Exerc.* 1990;22:436-43.
9. **Hughes C 4th, Hasselman CT, Best TM, Martinez S, Garrett WE Jr.** Incomplete, intrasubstance strain injuries of the rectus femoris muscle. *Am J Sports Med.* 1995;23:500-6.
10. **Jarvinen M, Sorvari T.** Healing of a crush injury in rat striated muscle. 1. Description and testing of a new method of inducing a standard injury to the calf muscles. *Acta Path Microbiol Scand [A].* 1975;83:259-65.
11. **Kasemkijwattana C, Menetrey J, Day CS, Bosch P, Buranapanitkit B, Moreland MS, Fu FH, Watkins SC, Huard J.** Biologic intervention in muscle healing and regeneration. *Sports Med Arthroscopy Rev.* 1998;6:95-102.
12. **Kasemkijwattana C, Menetrey J, Somogyi G, Moreland MS, Fu FH, Buranapanitkit B, Watkins SC, Huard J.** Development of approaches to improve the healing following muscle contusion. *Cell Transplant.* 1998;7:585-98.
13. **Kasemkijwattana C, Menetrey J, Bosch P, Somogyi G, Moreland MS, Fu FH, Buranapanitkit B, Watkins SS, Huard J.** Use of growth factors to improve muscle healing after strain injury. *Clin Orthop.* 2000;370:272-85.
14. **Lee CW, Fukushima K, Usas A, Xin L, Pelinkovich D, Martinek V, Somogyi G, Robbins PD, Fu FH, Huard J.** Biological intervention based on cell and gene therapy to improve muscle healing after laceration. *J Musculoskeletal Res.* 2000;4:265-77.
15. **Menetrey J, Kasemkijwattana C, Fu FH, Moreland MS, Huard J.** Suturing versus immobilization of a muscle laceration. A morphological and functional study in a mouse model. *Am J Sports Med.* 1999;27:222-9.
16. **Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, Moreland MS, Huard J.** Growth factors improve muscle healing in vivo. *J Bone Joint Surg Br.* 2000;82:131-7.
17. **Miller KJ, Thaloor D, Matteson S, Pavlath GK.** Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. *Am J Physiol Cell Physiol.* 2000;278:C174-81.
18. **Nikolaou PK, Macdonald BL, Glisson RR, Seaber AV, Garrett WE Jr.** Biomechanical and histological evaluation of muscle after controlled strain injury. *Am J Sports Med.* 1987;15:9-14.
19. **Jarvinen TA, Kaariainen M, Jarvinen M, Kalimo H.** Muscle strain injuries. *Curr Opin Rheumatol.* 2000;12:155-61.
20. **Best TM, Fiebig R, Corr DT, Brickson S, Ji L.** Free radical activity, antioxidant enzyme, and glutathione changes with muscle stretch injury in rabbits. *J Appl Physiol.* 1999;87:74-82.
21. **Lille ST, Lefler SR, Mowlavi A, Suchy H, Boyle EM, Farr AL Jr, Su CY, Frank N, Mulligan DC.** Inhibition of the initial wave of NF-kappaB activity in rat muscle reduces ischemia/reperfusion injury. *Muscle Nerve.* 2001;24:534-41.
22. **St Pierre BA, Tidball JG.** Differential response of macrophage subpopulations to soleus muscle reloading after rat hindlimb suspension. *J Appl Physiol.* 1994;77:290-7.
23. **Hurme T, Kalimo H, Lehto H, Jarvinen M.** Healing of skeletal muscle injury: an ultrastructural and immunohistochemical study. *Med Sci Sports Exerc.* 1991;23:801-10.
24. **Honda H, Kimura H, Rostami A.** Demonstration and phenotypic characterization of resident macrophages in rat skeletal muscle. *Immunology.* 1990;70:272-7.
25. **Aronson D, Wojtaszewski JF, Thorell A, Nygren J, Zangen D, Richter EA, Ljungqvist O, Fielding RA, Goodyear LJ.** Extracellular-regulated protein kinase cascades are activated in response to injury in human skeletal muscle. *Am J Physiol.* 1998;275(2 Pt 1):C555-61.
26. **St Pierre Schneider B, Correia LA, Cannon JG.** Sex differences in leukocyte invasion in injured murine skeletal muscle. *Res Nurs Health.* 1999;22:243-50.
27. **Pimorady-Esfahani A, Grounds MD, McMenamin PG.** Macrophages and dendritic cells in normal and regenerating murine skeletal muscle. *Muscle Nerve.* 1997;20:158-66.
28. **Tidball JG.** Inflammatory cell response to acute muscle injury. *Med Sci Sports Exerc.* 1995;27:1022-32.
29. **Cannon JG, St Pierre BA.** Cytokines in exertion-induced skeletal muscle injury. *Mol Cell Biochem.* 1998;179:159-67.
30. **Altstaedt J, Kirchner H, Rink L.** Cytokine production of neutrophils is limited to interleukin-8. *Immunology.* 1996;89:563-8.
31. **Best TM, Hunter KD.** Muscle injury and repair. *Phys Med Rehabil Clin N Am.* 2000;11:251-66.
32. **Li Y, Cummins J, Huard J.** Muscle injury and repair. *Curr Opin Orthop.* 2001;12:409-15.
33. **Russell B, Dix DJ, Haller DL, Jacobs-El J.** Repair of injured skeletal muscle: a molecular approach. *Med Sci Sports Exerc.* 1992;24:189-96.
34. **Trippel SB, Coutts RD, Einhorn TA.** Growth factors as therapeutic agents. *J Bone Joint Surg Am.* 1996;78:1272-86.
35. **Allen RE, Boxhorn LK.** Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor-I, and fibroblast growth factor. *J Cell Physiol.* 1989;138:311-5.
36. **Anderson JE, Liu L, Kardami E.** Distinctive patterns of basic fibroblast growth factor (bFGF) distribution in degenerative and regenerating areas of dystrophic (mdx) striated muscle. *Dev Biol.* 1991;147:96-109.
37. **Anderson JE.** Murray L. Barr Award Lecture. Studies of the dynamics of skeletal muscle regeneration: the mouse came back. *Biochem Cell Biol.* 1998;76:13-26.
38. **Barnard W, Bower J, Brown MA, Murphy M, Austin L.** Leukemia inhibitory factor (LIF) infusion stimulates skeletal muscle regeneration after injury: injured muscle expresses LIF mRNA. *J Neurol Sci.* 1994;123:108-13.
39. **Chambers RL, McDermott JC.** Molecular basis of skeletal muscle regeneration. *Can J Appl Physiol.* 1996;21:155-84.
40. **Doumit ME, Cook DR, Merkel RA.** Fibroblast growth factor, epidermal growth factor, insulin-like growth factors, and platelet-derived growth factor-BB stimulate proliferation of clonally derived porcine myogenic satellite cells. *J Cell Physiol.* 1993;157:326-32.
41. **Floss T, Arnold HH, Braun T.** A role for FGF-6 in skeletal muscle regeneration. *Genes Dev.* 1997;11:2040-51.
42. **Florini JR, Roberts AB, Ewton DZ, Falen SL, Flanders KC, Sporn MB.** Transforming growth factor-beta. A very potent inhibitor of myoblast differentiation, identical to the differentiation inhibitor secreted by Buffalo rat liver cells. *J Biol Chem.* 1986;261:16509-13.
43. **Gospodarowicz D, Weseman J, Moran JS, Lindstrom J.** Effect of fibroblast growth factor on the division and fusion of bovine myoblasts. *J Cell Biol.* 1976;70:395-405.
44. **Grounds MD.** Towards understanding skeletal muscle regeneration. *Path Res Pract.* 1991;187:1-22.
45. **Harrington MA, Daub R, Song A, Stasek J, Garcia JG.** Interleukin 1 alpha mediated inhibition of myogenic terminal differentiation: increased sensitivity of Ha-ras transformed cultures. *Cell Growth Diff.* 1992;3:241-8.
46. **Jennische E, Hansson HA.** Regenerating skeletal muscle cells express insulin-like growth factor I. *Acta Physiol Scand.* 1987;130:327-32.
47. **Jennische E.** Sequential immunohistochemical expression of IGF-I and the transferin receptor in regenerating rat muscle in vivo. *Acta Endocrinol (Copenh).* 1989;121:733-8.
48. **Jin P, Rahm M, Claesson-Welsh L, Heldin CH, Sejersen T.** Expression of PDGF A-chain and beta-receptor genes during rat myoblast differentiation. *J Cell Biol.* 1990;110:1665-72.
49. **Johnson SE, Allen RE.** Activation of skeletal muscle satellite cells and the role of fibroblast growth factor receptors. *Exper Cell Res.* 1995;219:449-53.
50. **Keller HL, St Pierre Schneider B, Eppihimer LA, Cannon JG.** Association of IGF-I and IGF-II with myofiber regeneration in vivo. *Muscle Nerve.* 1999;22:347-54.

51. **Kurek JB, Bower JJ, Romanella M, Koentgen F, Murphy M, Austin L.** The role of leukemia inhibitory factor in skeletal muscle regeneration. *Muscle Nerve.* 1997;20:815-22.

52. **Lefaucheur JP, Sebille A.** Muscle regeneration following injury can be modified in vivo by immune neutralization of basic fibroblast growth factor, transforming growth factor beta 1 or insulin-like growth factor I. *J Neuroimmunol.* 1995;57:85-91.

53. **Linkhart TA, Clegg CH, Hauschika SD.** Myogenic differentiation in permanent clonal mouse myoblast cell lines: regulation by macromolecular growth factors in the culture medium. *Dev Biol.* 1981;86:19-30.

54. **Lyles JM, Amin W, Bock E, Weill CL.** Regulation of NCAM by growth factors in serum-free myotube cultures. *J Neurosci Res.* 1993;34:273-86.

55. **McFarland DC, Pesall JE, Gilkerson KK.** The influence of growth factors on turkey embryonic myoblasts and satellite cells in vitro. *Gen Comp Endocrinol.* 1993;89:415-24.

56. **Gal-Levi R, Leshem Y, Akoi S, Nakamura T, Halevy O.** Hepatocyte growth factor plays a dual role in regulating skeletal muscle satellite cell proliferation and differentiation. *Biochim Biophys Acta.* 1998;1402:39-51.

57. **Olson EN, Sternberg E, Hu JS, Spizz G, Wilcox C.** Regulation of myogenic differentiation by type beta transforming growth factor. *J Cell Biol.* 1986; 103:1799-805.

58. **Yablonka-Reuvini Z, Balestrer TM, Bowen-Pope DF.** Regulation of proliferation and differentiation of myoblasts derived from adult mouse skeletal muscle by specific isoforms of PDGF. *J Cell Biol.* 1990;111:1623-9.

59. **Damon SE, Haugk KL, Birnbaum RS, Quinn LS.** Retrovirally mediated overexpression of insulin-like growth factor binding protein 4: evidence that insulin-like growth factor is required for skeletal muscle differentiation. *J Cell Physiol.* 1998;175:109-20.

60. **Engert JC, Berglund EB, Rosenthal N.** Proliferation precedes differentiation in IGF-I stimulated myogenesis. *J Cell Biol.* 1996;135:431-40.

61. **Florini JR, Ewton DZ, Falen SZ, Van Wyk JJ.** Biphasic concentration dependency of stimulation of myoblast differentiation by somatomedins. *Am J Physiol.* 1986;250:C771-8.

62. **Quinn LS, Haugk KL.** Overexpression of the type-1 insulin-like growth factor receptor increases ligand-dependent proliferation and differentiation in bovine skeletal myogenic cultures. *J Cell Physiol.* 1996;168:34-41.

63. **Florini JR, Ewton DZ, Coolican SA.** Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev.* 1996;17:481-517.

64. **Zdanowicz MM, Moyse J, Wingertzahn MA, O'Connor M, Teichberg S, Slonim AE.** Effect of insulin-like growth factor I in murine muscular dystrophy. *Endocrinology.* 1995;136:4880-6.

65. **Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C, Schwartz RJ.** Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *J Biol Chem.* 1995;270:12109-16.

66. **Papadakis MA, Grady D, Black D, Tierney MJ, Gooding GA, Schambelan M, Grunfeld C.** Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann Intern Med.* 1996;124: 708-16.

67. **Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL.** Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc Natl Acad Sci U S A.* 1998;95:15603-7.

68. **Lamberts SW, van den Beld AW, van der Lely AJ.** The endocrinology of aging. *Science.* 1997;278:419-24.

69. **Jones JI, Clemmons DR.** Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev.* 1995;16:3-34.

70. **Sheehan SM, Tatsumi R, Temm-Grove CJ, Allen RE.** HGF is an autocrine growth factor for skeletal muscle satellite cells in vitro. *Muscle Nerve.* 2000;23:239-45.

71. **Sheehan SM, Allen RE.** Skeletal muscle satellite cell proliferation in response to members of the fibroblast growth factor family and hepatocyte growth factor. *J Cell Physiol.* 1999;181:499-506.

72. **Tatsumi R, Anderson JE, Nevoret CJ, Halevy O, Allen RE.** HGF/SF is present in normal adult skeletal muscle and is capable of activating satellite cells. *Dev Biol.* 1998;194:114-28.

73. **Springer ML, Chen AS, Kraft PE, Bednarski M, Blau HM.** VEGF gene delivery to muscle: potential role for vasculogenesis in adults. *Mol Cell.* 1998;2:549-58.

74. **Gowdak LH, Poliakova L, Wang X, Kovesdi I, Fishbein KW, Zacheo A, Palumbo R, Straino S, Emanueli C, Marrocco-Trischitta M, Lakatta EG, Anversa P, Spencer RG, Talan M, Capogrossi MC.** Adenovirus-mediated VEGF(121) gene transfer stimulates angiogenesis in normoperfused skeletal muscle and preserves tissue perfusion after induction of ischemia. *Circulation.* 2000;102:565-71.

75. **van Deutekom JC, Floyd SS, Booth DK, Oligino T, Krisky D, Marconi P, Glorioso JC, Huard J.** Implications of maturation for viral gene delivery to skeletal muscle. *Neuromuscular Disord.* 1998;8:135-48.

76. **van Deutekom JC, Hoffman EP, Huard J.** Muscle maturation: implications for gene therapy. *Mol Med Today.* 1998;4:214-20.

77. **Labhasetwar V, Bonadio J, Goldstein S, Chen W, Levy RJ.** A DNA controlled-release coating for gene transfer: transfection in skeletal and cardiac muscle. *J Pharm Sci.* 1998;87:1347-50.

78. **Appleton I, Tomlinson A, Colville-Nash PR, Willoughby DA.** Temporal and spatial immunolocalization of cytokines in murine chronic granulomatous tissue. Implications for their role in tissue development and repair processes. *Lab Invest.* 1993;69:405-14.

79. **Barcellos-Hoff MH, Derynck R, Tsang ML, Weatherbee JA.** Transforming growth factor-beta activation in irradiated murine mammary gland. *J Clin Invest.* 1994;93:892-9.

80. **Barnes JL, Abboud HE.** Temporal expression of autocrine growth factors corresponds to morphological features of mesangial proliferation in Habu snake venom-induced glomerulonephritis. *Am J Pathol.* 1993;143:1366-76.

81. **Brandes ME, Allen JB, Ogawa Y, Wahl SM.** Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals. *J Clin Invest.* 1991;87:1108-13.

82. **Coimbra T, Wiggins R, Noh JW, Merritt S, Phan SH.** Transforming growth factor-beta production in anti-glomerular basement membrane disease in the rabbit. *Am J Pathol.* 1991;138:223-34.

83. **Czaja MJ, Weiner FR, Flanders KC, Giambrone MA, Wind R, Biempica L, Zern MA.** In vitro and in vivo association of transforming growth factor-beta 1 with hepatic fibrosis. *J Cell Biol.* 1989;108:2477-82.

84. **Jones CL, Buch S, Post M, McCulloch L, Liu E, Eddy AA.** Pathogenesis of interstitial fibrosis in chronic purine aminonucleoside nephrosis. *Kidney Int.* 1991;40:1020-31.

85. **Kagami S, Border WA, Miller DE, Noble NA.** Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. *J Clin Invest.* 1994;93:2431-7.

86. **Kaneto H, Morrissey J, Klahr S.** Increased expression of TGF-beta 1 mRNA in the obstructed kidney of rats with unilateral ureteral ligation. *Kidney Int.* 1993;44:313-21.

87. **Khalil N, Whitman C, Zuo L, Danielpour D, Greenberg A.** Regulation of alveolar macrophage transforming growth factor-beta secretion by corticosteroids in bleomycin-induced pulmonary inflammation in the rat. *J Clin Invest.* 1993;92:1812-8.

88. **Logan A, Berry M, Gonzalez AM, Frautschy SA, Sporn MB, Barid A.** Effects of transforming growth factor beta 1 on scar production in the injured central nervous system of the rat. *Eur J Neurosci.* 1994;6:355-63.

89. **Okuda S, Languino LR, Ruoslahti E, Border WA.** Elevated expression of transforming growth factor-beta and proteoglycan production in experimental glomerulonephritis. Possible role in expansion of the mesangial extracellular matrix. *J Clin Invest.* 1990;86:453-62.

90. **Sporn MB, Roberts AB.** A major advance in the use of growth factors to enhance wound healing. *J Clin Invest.* 1993;92:2565-6.

91. **Terrell TG, Working PK, Chow CP, Green JD.** Pathology of recombinant human transforming growth factor-beta 1 in rats and rabbits. *Int Rev Exp Pathol.* 1993;34:43-67.

92. **Westergren-Thorsson G, Hernnas J, Sarnstrand B, Oldberg A, Heinegard D, Malmstrom A.** Altered expression of small proteoglycans, collagen, and transforming growth factor-beta 1 in developing bleomycin-induced pulmonary fibrosis in rats. *J Clin Invest.* 1993;92:632-7.

93. **Williams RS, Rossi AM, Chegini N, Schultz G.** Effect of transforming growth factor beta on postoperative adhesion formation and intact peritoneum. *J Surg Res.* 1992;52:65-70.

94. **Wolf YG, Rasmussen LM, Ruoslahti E.** Antibodies against transforming growth factor-beta 1 suppress intimal hyperplasia in a rat model. *J Clin Invest.* 1994;93:1172-8

95. **Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA.** Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci U S A.* 1993;90:1814-8.

96. **Yamamoto T, Noble NA, Miller DE, Border WA.** Sustained expression of TGF-beta 1 underlies development of progressive kidney fibrosis. *Kidney Int.* 1994;45:916-27.

97. **Bernasconi P, Torchiana E, Confalonieri P, Brugnoni R, Barresi R, Mora M, Cornelio F, Morandi L, Mantegazza R.** Expression of transforming growth factor-beta 1 in dystrophic patient muscles correlates with fibrosis. Pathogenetic role of a fibrogenic cytokine. *J Clin Invest.* 1995;96:1137-44.

98. **Yamazaki M, Minota S, Sakurai H, Miyazono K, Yamada A, Kanazawa I, Kawai M.** Expression of transforming growth factor-beta 1 and its relation to endomysial fibrosis in progressive muscular dystrophy. *Am J Pathol.* 1994;14:221-6.

99. **Amemiya K, Semino-Mora C, Granger RP, Dalakas MC.** Downregulation of TGF-beta 1 mRNA and protein in the muscles of patients with inflammatory myopathies after treatment with high-dose intravenous immunoglobulin. *Clin Immunol.* 2000;94:99-104.

100. **Confalonieri P, Bernasconi P, Cornelio F, Mantegazza R.** Transforming growth factor-beta 1 in polymyositis and dermatomyositis correlates with fibrosis but not with mononuclear cell infiltrate. *J Neuropathol Exp Neurol.* 1997;56:479-84.

101. **Tredget EE, Wang R, Shen Q, Scott PG, Ghahary A.** Transforming growth factor-beta mRNA and protein in hypertrophic scar tissues and fibroblasts: antagonism by IFN-alpha and IFN-gamma in vitro and in vivo. *J Interferon Cytokine Res.* 2000;20:143-51.

102. **Zhang L, Mi J, Yu Y, Yao H, Chen H, Li M, Cao X.** IFN-gamma gene therapy by intrasplenic hepatocyte transplantation: a novel strategy for reversing hepatic fibrosis in Schistosoma japonicum-infected mice. *Parasite Immunol.* 2001;23:11-7.

103. **Gurujeyalakshmi G, Giri SN.** Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. *Exp Lung Res.* 1995;21:791-808.

104. **Oldroyd SD, Thomas GL, Gabbiani G, El Nahas AM.** Interferon-gamma inhibits experimental renal fibrosis. *Kidney Int.* 1999;56:2116-27.

105. **Mietz H, Krieglstein GK.** Suramin to enhance glaucoma filtering procedures: a clinical comparison with mitomycin. *Ophthalmic Surg Lasers.* 2001;32:358-69.

106. **Kloen P, Jennings CL, Gebhardt MC, Springfield DS, Mankin HJ.** Suramin inhibits growth and transforming growth factor-beta 1 (TGF-beta 1) binding in osteosarcoma cell lines. *Eur J Cancer.* 1994;30:678-82.

107. **Zein NN.** Interferons in the management of viral hepatitis. *Cytokines Cell Mol Ther.* 1998;4:229-41.

108. **Friedlander L, van Thiel DH, Faruki H, Molloy PJ, Kania RJ, Hassanein T.** New approach to HCV treatment. Recognition of disease process as systemic viral infection rather than as liver disease. *Dig Dis Sci.* 1996; 41:1678-81.