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1. Introduction

This book chapter is to further improve our understanding of the underlying mechanism of local injury that occurs after manual acupuncture needle manipulation that initiates muscle degeneration/regeneration process, which is essential for muscle maintenance and adaptation. Muscle injury triggers a sequence of events that begins with the host inflammation, secretion of myogenic transcription factor and various growth factors that are important for the activation and proliferation of muscle satellite (stem) cells. Skeletal muscle is maintained by resident stem cells called muscle satellite cells because of their location on the periphery of the myofiber under the surrounding basal lamina (Mauro, 1961). Satellite cells are quiescent mononuclear cells that are present in different types of skeletal muscle and are associated with all muscle fiber types, although the distribution might be unequal. For instance, the percentage of satellite cell in adult tibialis anterior muscle is larger than other muscles (Pavlath et al., 1998). Satellite cells are responsible for postnatal growth, hypertrophy and repair of skeletal muscle (Grounds, 1999). In normal undisturbed muscle, these cells remain in a quiescent stage (Schultz et al., 1985). After muscle injury, satellite cells become mitotically active, proliferate, differentiate and eventually fuse together to regenerate new muscle fiber, which is well-innervated, fully vascularized, through a process that resembles in some aspects embryonic (Seale et al., 2001). During muscle differentiation, satellite cells exhibit increased expression of the myogenic transcription factor MyoD (Cooper et al., 1999). Analysis of muscle regeneration has previously been studied using experimental models that induce muscle injury by various protocol such as eccentric contraction (Rathbone et al., 2003) or treadmill exercise (Smith et al., 2001). Although exercise induces injury-related satellite cell activation (Smith et al., 2001), its use is limited in patients with restricted exercise capacity during long periods of bed rest. Instead, such injury can be induced during acupuncture needle manipulation, which has been shown to induce muscle injury on acupuncture point (ST-36) in the tibialis anterior (TA) muscle (see Figure 1) and produce muscle regeneration via up-regulation of MyoD (see Figure 2 and Figure 3).
Fig. 1. Acupuncture needling-induced signs of muscle injury
Characteristic sagittal sections of hematoxylin-stained TA muscle. (A-left slide) Non-treated control TA muscle reveals a uniform labeling of compact myofibers and resident nuclei. (B-right slide) Acupuncture needling of the right TA muscle induced loss of myofiber integrity and apparent myonecrosis. Needling also stimulated the infiltration of mononucleated cells to the injured sites within the muscle tissue (arrow). Bar = 100 microns. (Ameis et al. 2008)

Fig. 2. Evidence of myogeneic potential with TA muscle. (A) Serial sections of control TA muscle incubated with antibodies specific for MyoD routinely displayed low levels of immunoreactivity. (B) MyoD-positive cells (arrows) were consistently observed in subsets of cells within the acupunctured TA muscle tissue. Immunoreactive profiles identified dense, nuclear staining that was localized at the periphery of the fiber membrane (arrows). Bar = 50 microns. (Ameis et al., 2008).
Degeneration/Regeneration as a Mechanism Contributing to the Effect of Manual Acupuncture-Induced Injury on Rat Skeletal Muscle

2. Acupuncture and skeletal muscle regeneration

Acupuncture is an ancient Chinese therapy with a mode of action that is controversial. In traditional Chinese medicine (TCM) theory, needle manipulation is an important element associated with the efficacy of manual acupuncture. The acupuncture needles are manually manipulated after their insertions into a specific acupuncture point. Needle manipulation typically consists of rapid back-and-forth rotation, which can be brief (a few seconds) or prolonged (several minutes). Traditionally, in humans, needle manipulation is performed to elicit the characteristic reaction of acupuncture needling known as de’qi (Park et al., 2002). De’qi has a sensory component that is perceived by the patient as a pinching-type pain or heaviness in the area surrounding the needle. The acupuncturist administering treatment...

Western Blot Analysis

Fig. 3. Acupuncture needling-induced MyoD protein level as determined by Western Blot analysis. (A) The gel image of MyoD and beta-actin in control and treated TA muscle tissue. Lane (1, 2, 3 and 4 represent the different leg muscles) (B) Relative optical density. The results indicated a significant increase in treated compared with control (*** p<0.001) using Student t test. (Ameis et al., 2008)
perceives de’qi by needle grasp, or resistance to further manipulation. De’qi is widely viewed as essential to acupuncture’s therapeutic effectiveness. It is possible that the de’qi reaction is a sign of muscle fiber injury or degeneration with subsequent regeneration.

3. Muscle injury and the degenerative phase

The degenerative phase of skeletal muscle injury involves inflammatory cells which is a critical component for successful regeneration to occur (Chargé & Rudnicki, 2004). Among inflammatory cells is a macrophages that infiltrate the injury site to phagocytose muscle debris (Robertson et al., 1993) and release interleukin-6 (IL-6) (Allen et al., 1995). IL-6 has the potential to regulate many critical process related to skeletal muscle including the regulation of cell proliferation, cell differentiation and overall tissue regeneration response (Baeza-Raja & Muñoz-Cánoves, 2004; Cantini et al., 1995; Kami et al., 2000). It has been shown that IL-6 plays an essential role in regulation of satellite cell (Serrano et al., 2008). Loss of IL-6 results in reduced satellite cell proliferation and migration (Serrano et al., 2008). Although IL-6 is classified as a pro-inflammatory cytokine it has a variety of biological functions. IL-6 can also act as in anti-inflammatory manner to inhibit the inflammatory response (Bunn et al., 2004; Xing et al., 1998).

The expression of IL-6 mRNA was detected in different injury models of muscle regeneration (Balasubramaniam et al., 2009; Sheriff et al., 2009; Warren et al., 2002). For example, Bunn et al. (2004) investigated the increase of IL-6 during regeneration following crush injury in rat muscle at different time points: 2, 4, 8, 16, and 24 days. The results showed a high level increase on day 2 post injury, decrease on day 4 and then increase again on day 8. Our current, unpublished data using acupuncture induced injury showed the expression of IL-6 mRNA was elevated rapidly in response to manual acupuncture in the treated rats TA muscle than the control at day 1. The expression was decreased at day 3 and increased again at day 7. It decreased again at day 21 post treatment (see Figure 4). Taken together, the results of the studies discussed show that the inflammatory response seen after acupuncture injury is in many ways similar to those reported after other types of skeletal muscle injury. However acupuncture-induced model of injury has the added benefit that it could be applied in humans to study the recovery process following injury. Muscle injury common in contact sport has been studied in animal models and recently particular attention has been paid to skeletal muscle regeneration after injury. Attention has also focused on various treatment modalities in order to speed up the recovery process as well as to facilitate anti-inflammatory actions.

Although it is known that the immune system plays a major role in the skeletal muscle regeneration after injury, little detailed research has been done on the immune components of physiological response to injury so that conclusions are currently quite generalized or even conflicting. This makes it difficult to suggest incontrovertible evidence-based recommendations for treatment. Acupuncture has been accepted as a technique which can be used in humans, in contrast to other muscle-injury models (such as injection of toxins, freezing or crushing).

4. Muscle injury and regeneration

The regeneration phase, in which muscle stem cell are activated and rapidly up-regulate the expression of myogenic regulatory factor (MyoD). This cellular and molecular phenotype is a
Degeneration/Regeneration as a Mechanism Contributing to the Effect of Manual Acupuncture-Induced Injury on Rat Skeletal Muscle

Fig. 4. **IL-6 Expression** Data represent fold changes in *IL-6* mRNA as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (19.44, 4.74, 3.53, 1.23 folds) different from control. (unpublished data, Ameis et al.)

presumed prerequisite for muscle regeneration and repair. At the molecular level, activation of satellite cells is characterized by the expression of MyoD (Cooper et al., 1999), which is routinely associated with cell determination, and is often highly expressed in actively proliferating myoblasts. Moreover, MyoD expression may be critical for the differentiation of myoblasts in response to stimuli that are produced during muscle injury (Seale and Rudnicki, 2002; Sabourin and Rudnicki, 2000) and remodeling. The absence of MyoD adversely affects muscle regeneration, delaying the transition of satellite cell-derived myoblast from proliferation to differentiation (Yablonka-Reuveni et al., 1999). Increased expression of MyoD has been shown to occur in both adult and aged skeletal muscle in response to injury (Becker et al., 2003; Krainak et al., 2006). It has been expressed also in young skeletal muscle following manual acupuncture treatment applied in acupuncture point ST36, located at the tibialis anterior (TA) muscle (Ameis et al., 2008).

The capacity of muscle regeneration is related to several factors, including the available pool of resident satellite cells in muscle fiber. TA muscle reportedly contains a greater pool of resident satellite cells than other muscle tissues (Kadi et al., 2004; Pavlath et al. 1998) and TA muscle regeneration and remodeling after injury is initiated at faster rates (Pavlath et al. 1998). These characteristics of the TA muscle are most likely related to its functional demand; TA muscle helps coordinate the control of balance and foot stability during normal walking, and is therefore active for extended periods daily. ST36 is the most commonly targeted acupuncture needle point in both clinical settings (Li et al., 2004), and experimental studies (Ameis, 1991).
Several pathways, including insulin-like growth factor (IGF-1) play vital role in regulating muscle regeneration (Machida & Booth, 2004). IGF-1 is a member of the family of insulin-related peptides. It is a widely distributed trophic hormone capable of mediating autocrine, paracrine or endocrine effect. IGF-1 was originally referred to as “somatomedin” to reflect its expression response to growth hormone stimulation and its ability to mediate some of the growth-promoting effect of growth hormones (de Moor et al., 1994). IGF-1 plays important roles in both embryonic and post-natal mammalian growth (van Buul-Offers et al., 1994). Early studies compared the metabolic effect of IGF-1 and insulin on muscles including stimulation of protein metabolism, glucose transport and glycogen and triglyceride synthesis. IGF-1 has been associated specifically with increasing proliferation of satellite cells (Hill & Goldspink, 2003). IGF-1 has been showed to be increased following acute muscle damage or chronic aerobic exercise (Bamman et al., 2001; Hambrecht et al., 2005). Several pathways, including IGF-1 regulate muscle regeneration. IGF-1 has been specifically associated with increasing proliferation of satellite cells (Machida & Booth 2004). The local up-regulation of IGF-1 mRNA has been induced in skeletal muscle by various models of muscle injury, such as myotoxin injection (Hill & Goldspink, 2003; Keller et al., 1999; McKoy et al. 1999) or eccentric contraction (Hill & Goldspink, 2003; Hill et al., 2003; Jennische & Hansson, 1987). In our published study using acupuncture-induced injury, IGF-1 was higher in the TA muscle after manual acupuncture treatment than control (see Figure 5). IGF-1 remained higher than control at day 1 of post acupuncture treatment and peaked at day 3. On day 7 and day 21, IGF-1 had started to return to basal normal.

![IGF-1 Expression](https://www.intechopen.com)

**Fig. 5. IGF-1** Data represent fold changes in IGF-1 as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (0.6, 0.31, 0.96, 0.19 folds) different from control. (unpublished data, Ameis et al.)
Degeneration/Regeneration as a Mechanism Contributing to the Effect of Manual Acupuncture-Induced Injury on Rat Skeletal Muscle

5. Acupuncture as a model of muscle injury

After pathological or experimental injury, the muscle undergoes regeneration processes. This regenerative capacity is due to the presence of a tissue-specific population of myogenetic stem cells. These specialized somatic stem cells are termed satellite cell because of their location on the periphery of the myofiber under the surrounding basal lamina (Mauro, 1961). During muscle development, satellite cells also mediate the post natal growth and are the primary means by which the mass of adult muscle is formed (Grounds, 1999). During post natal muscle development, satellite cells fuse with new or pre-existing myofiber. In adult muscle, in response to stress induced by weight-bearing exercise or trauma, satellite cells become mitotically active, proliferate, differentiate, and eventually fuse together to regenerate new muscle fiber (Seale et al., 2001; Chargé and Rudnicki, 2004). The essential role of satellite cells in muscle regeneration is well documented. However, an understanding of the molecular mechanism that regulates the activation and foundation of muscle stem cells has remained elusive and is a topic of extensive research targeting the crucial involvement of satellite cells in muscle reconstruction.

At the molecular level, activation, proliferation and differentiation of satellite cells is characterized by the up regulation of myogenic regulatory factor (MRFs). MRFs are skeletal muscle-specific transcription factor. The family consists of MyoD, Myogenin, MYF-5, MRF-4 to a larger basic-helix-loop-helix (bHLH) class of transcription factor. MyoD has been shown to up-regulate at the early stage of satellite cell proliferation. Myogenin and MRF-4 is up-regulated in cells, beginning their terminal differentiation program (Chargé and Rudnicki, 2004, review).

Analysis of muscle regeneration has previously been reported using different treatments to first induce degeneration of the muscle tissue, such as freezing (Warren et al., 2002) or snake toxins (such as notexin or cardiotoxin) (Mendler et al., 1998; Yan et al., 2003). Instead, such muscle injury can be induced using acupuncture, a minimally invasive technique. A study showed that a single session of manual acupuncture, consisting of needle manipulation in rat TA muscle, lead to focal injury and activation of satellite cell via up-regulated MyoD protein within muscle fiber (Ameis et al., 2008). A recent unpublished study has shown that manual acupuncture-induced injury in rat TA muscle up-regulated the expression of myogenic regulatory factor MyoD mRNA and peaked at 24 h post manual acupuncture treatment (see Figure 6). MyoD mRNA expression that peaked at 24 h has also been reported in rat pantails and soleus muscle in response to mechanical overload and stretch (Owino et al., 2001; Peviani et al., 2007).

In contrast to those using non-physiological models of muscle damage, other injury models have shown different time peak of MyoD. For example, Mendler et al. (1998) investigated the mRNA expression of MyoD during muscle regeneration following injection of myotoxin in rat muscle found an increase of MyoD mRNA at 72 h post injury induced by injection of these myotoxin in rat muscle. Yan et al. (2003) using the same injury model using snake venom extract observed similar peak time point at 72h post injury in mice muscle. The reason for the difference in the time course of expression of MyoD mRNA between our study and those using venom toxin is unclear, but may be related to the severity of the insult that may lead to a partial loss of the satellite cell population (Polesskaya et al., 2003). However, whether this holds true is a topic for further investigation.

An alternative method to myotoxin which is considered more physiologically relevant is freeze injury (Chargé and Rudnicki, 2004). It has been shown that after freezing injury,
MyoD mRNA expression increased in mice TA muscle and peaked at 72 h time point (Warren et al., 2002).

Fig. 6. MyoD1 Data represent fold changes in MyoD1 mRNA as assessed by RT-PCR after 0 (control), 1, 3, 7 and 21 days post acupuncture treatment of TA muscle. Differences in expression between groups were assessed by two-sided Pair Wise Fixed Reallocation Randomization Test. Level of probability was set at $P < 0.05$ as statistically significant. Values are mean +/- SEM (0.00, 17.88, 5.62, 0.34 folds) different from control. (unpublished data, Ameis et al.)

Comparing the effect of eccentric contraction and freezing shows that eccentric contraction exhibits a higher expression of MyoD mRNA than freezing (Warren et al., 2007). This might be explained by the observation that freezing induced freezing of satellite cells (Irintchev et al., 1997; Pastoret and Partridge, 1998).

The earlier peak expression of MyoD mRNA may be due to the nature of the intervention in the acupuncture needling model of muscle injury, which is similar to mechanical overload and stretch. The intervention involves a single insertion of a needle into acupuncture point ST36 in the rat TA muscle followed by a slight back-and-forth manipulation of the needle. During acupuncture needle manipulation, the muscle would be stretched and for normal muscle it is enough to hold a stretch for 15 to 30 seconds and repeat it for 3 to 5 times (Pezzullo & Irrgang, 2001). Therefore acupuncture needling model of injury, the needle is manipulated for 20 seconds every 3 minutes over duration of 15 minutes total. Therefore, this technique induces immediate and distant muscle injury--the needle insertion causes focal muscle injury at the point of insertion and the needle manipulation which stretches muscle fiber causes more distant muscle fiber injury.

There are several potential research advantages of acupuncture induced injury versus mechanical overload and stretch. The acupuncture injury model can be used to understand the process of muscle regeneration that will allow more precise comparison in future investigations of regenerative differences between age groups. It can also be used to study the regenerative process. Knowledge of the sets of genes associated specifically with the
nature of the injury may have application for developing new strategies for acceleration of the recovery process in injury skeletal muscle.

6. Possible role of acupuncture used as a countermeasure to forced inactivity

Forced inactivity during bed rest (Fitts et al., 1986) and space flight (Taylor et al., 2002) result in decrease of muscle regeneration. During activity, skeletal muscles are damaged and repaired repeatedly throughout life. Muscle regeneration maintains locomotor function during aging and delays the appearance of clinical symptoms of several conditions of muscle wasting. In humans, satellite cell function declines during aging (Gnocchi et al. 2008). However, in response to exercise, satellite cells increase in both young and old men and women, with significant increase particularly in adults (Dreyer et al., 2006; Kadi et al., 2004). In aged rats, satellite cells displayed a longer lag period before entering the cell cycle compared to adult rats (Gopinath and Rand o, 2008). During inactivity (ex. hind limb unloading), the satellite cell mitotic activity is significantly reduced, especially in aged rats (Darr and Schultz 1989; Mozdziak et al., 2000; Siu et al., 2005). Therefore, it is suggested that activation of satellite cell may inhibit the loss of muscle function under atrophic conditions. Prevention of skeletal muscle atrophy during prolonged inactivity is important. A variety of potential counter measures have been tested and in many cases proved ineffective or unsatisfactory. In more recent study, daily injection of the complex IGF-1 during hind limb suspension or recovery showed to be beneficial (Chakravarthy et al., 2000; Zdanowica & Teichberg, 2003).

Because of the importance of IGF-1 to muscle regeneration, therapeutic applications are beginning to be explored. For example, experimenton on aged rat recovering from hindlimb immobilization shows that exogenously administration IGF-1 stimulates muscle recovery and an increase in satellite cell proliferation potential in hindlimb musculature (Chakravarthy et al., 2000). IGF-1 has been naturally expressed following manual acupuncture, therefore we suggest that acupuncture may be used as an approach to enhance muscle regeneration during forced inactivity such as bed rest.

This book chapter has sought to enhance our understanding of the underlying mechanism of local injury that occurs after manual acupuncture needle manipulation that initiates the degeneration/regeneration process. Muscle injury induced by acupuncture needling triggers a sequence of events that begins with the host inflammation, secretion of myogenic transcription facto and various growth factors that are important for the activation and proliferation of muscle satellite (stem) cells.

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Degeneration/Regeneration as a Mechanism Contributing to the Effect of Manual Acupuncture-Induced Injury on Rat Skeletal Muscle

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Acupuncture and related techniques are useful tools for treating a spectrum of diseases. However, there are still many areas of controversy surrounding it. We hope this book can contribute to guide the advance of this ancient medical art. In the present work, the reader will find texts written by authors from different parts of the world. The chapters cover strategic areas to collaborate with the consolidation of the knowledge in acupuncture. The book doesn’t intend to solve all the questions regarding this issue but the main objective is to share elements to make acupuncture more and better understood at health systems worldwide.

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