Impact of inflammation and anti-inflammatory modalities on skeletal muscle healing: from fundamental research to the clinic.

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Abstract

Anti-inflammatory modalities are commonly used for the treatment of various musculoskeletal injuries. While inflammation was originally believed to interfere with skeletal muscle regeneration, several recent studies have highlighted the beneficial effects of inflammatory cells on muscle healing. This discrepancy is attributable to our evolving understanding of the complex inflammatory process. To better appreciate the paradoxical roles of inflammation, clinicians must have a better comprehension of the fundamental mechanisms regulating the inflammatory response. In this perspective paper, we analyzed cellular, animal and human studies to summarize recent knowledge regarding inflammation's impact on muscle regeneration in acute or chronic conditions. We also discussed the effect of anti-inflammatory drugs in the treatment of various muscle injuries. Overall, this work aims to summarize the current state of the literature on the inflammatory process associated with muscle healing in order to give clinicians the necessary tools to have a more efficient and evidence-based practice for the treatment of muscle injuries and disorders.

Introduction

The majority of physical therapists are confronted in their everyday work with different types of musculoskeletal injuries. One common feature for most of these injuries is the presence of an inflammatory response, which can be acute or chronic, mild or severe, and septic or sterile. Physiotherapists possess different physical, thermal, electrotherapeutic, and even molecular tools that dampen, to varying degrees of efficiency, the inflammatory response. Notably, in 2013, physiotherapists from the UK were the first to obtain the right to prescribe non-steroidal anti-inflammatory drugs (NSAIDs) (1). Physiotherapists from other countries, such as New-Zealand, Australia, Canada, United States and others, do not yet have the legislative right to prescribe NSAIDs or any other analgesics; nevertheless, a high proportion of physiotherapists will directly recommend NSAIDs or will emphasize to their patients the importance of seeking advice from their pharmacist or physician (2,3). However, multiple studies suggested that their knowledge on NSAIDs is incomplete or inadequate (3,4). The results from a survey targeting physiotherapists from different countries showed that most physiotherapists did not recently upgrade their knowledge on NSAIDs use (5). Another study on the perception of physiotherapists on NSAIDs showed that (1) the majority of physiotherapists believed that NSAIDs are more efficient than analgesic (acetaminophen) to relieve pain in musculoskeletal injuries, and (2) less than half of the physiotherapists believed that NSAIDs should be withheld during the first few days after an injury to avoid interfering with the beneficial effect of inflammation (3). These results reveal contradictory views on the efficacy of anti-inflammatory drugs among physiotherapists, which result from a lack of knowledge regarding the effect of inflammation on tissue healing. While the academic curricula recently changed in some countries to include additional information on the use of different anti-inflammatory drugs, we believe that an update of this fast-evolving field will bring novel and clinically important information to physiotherapists.

In a recent paper published in *Physical Therapy Journal*, Norland R *et al* indicate that a gap exists between basic science research and clinical practices (6). The authors

suggested that health professionals would benefit from integrating the notion of regenerative rehabilitation, which combines rehabilitation with regenerative medicine (such as biomaterial or cell therapies), to their practice in order to provide an optimal environment for tissue healing (6,7). For clinicians to do so, however, academics must ensure that the recent advances in fundamental biological sciences are accessible and can be implemented in a clinical setting. In agreement, we believe that to understand the efficacy of anti-inflammatory modalities, clinicians must first comprehend the fundamental nature and function of the inflammatory process. In this review, we describe the interactions between inflammatory cells and satellite cells, the latter of which are responsible for muscle regeneration (see table 1 for the description of the different cell types). We compare the effect of the inflammatory process in acute and chronic injury and analyze the efficacy of NSAIDs on muscle healing.

Muscle regeneration

Skeletal muscles constitute approximately 30-40% of total body mass and have many vital roles such as generation of movement, protection, breathing, thermoregulation and metabolism. Movement is generated by the contraction of long cylindrical cells named myofibers, which are the most important part of skeletal muscle composition (8). The integrity and the function of these cells can be affected by different traumas and conditions such as strains, contusions, lacerations, immobilisation, eccentric-induced muscle damage, ischemia and others. Because the nuclei of the myofibers are terminally post-mitotic (i.e. they cannot divide anymore), muscle regeneration is ensured by a population of adult muscle stem cells, named satellite cells (9). In absence of satellite cells, skeletal muscle regeneration post-injury is prevented (10,11). One exception is skeletal muscle hypertrophy (a process that does not affect the cellular integrity of myofibers), where an increase in myofiber size is possible in absence of satellite cells, although it plateaus faster and it is associated with signs of impaired healing such as fibrosis deposition (12,13). Overall, satellite cells are essential to muscle regeneration post-injury, and they also contribute to muscle hypertrophy.

Following an injury, satellite cells activate and become myoblasts that proliferate extensively for the first few days (Figure 1). Three to seven days after the injury, myoblasts stop proliferating to differentiate and either fuse to the damaged myofibers or fuse together to form myotubes (immature myofibers). During the next few weeks, these newly formed myofibers grow to form new mature myofibers. In murine models, this very efficient process explains the remarkable regenerative capacity of murine skeletal muscles, which can largely regain their integrity and function only a few weeks following a severe injury (14). While human skeletal muscles also have an important regenerative capacity, it might not be as efficient. Accordingly, it was shown that muscle morphology can still be altered long after an injury (15). Notably, both in animals and humans, muscle injury also activates an inflammatory response characterized by the coordinated recruitment of inflammatory cells to the site of injury (16). The onset, development, and resolution of the inflammatory process have a critical role on the guidance of satellite cell function and thus, on muscle regeneration (17).

The acute inflammatory process

While physiotherapy treatments aim at decreasing clinical signs of inflammation such as swelling, pain and loss of function, the cellular and molecular mechanisms governing the inflammatory process remain elusive for many. This could be partially explained by the fact that the definition of inflammation continues to evolve alongside the advances in modern molecular biology. Indeed, while inflammation was first defined solely based on clinical signs and symptoms, it is now known that it involves a broad spectrum of inflammatory conditions that are initiated by different stimuli and that activates a multitude of complex biological processes (18). Sterile inflammation, occurring in acute pathogen-free conditions such as sport injuries, has been extensively studied in the last decade, and a large body of evidence has emerged regarding the influence of inflammatory cells on the healing process. While human studies are more easily transferable to physical therapy practice, animal and cellular studies have allowed us to increase our understanding of inflammation by developing

several information-rich experimental models (16). In the next section, we will use these human, animal and cellular models of muscle injury to discuss the different phases of the inflammatory process, i.e. the onset, the development, and the dampening of inflammation.

Onset of the inflammatory process: Injury-induced disruption of blood vessel integrity has long been considered as the starting point of the inflammatory process by activating the blood coagulation cascade, which, among other roles, leads to the formation of anaphylatoxins. These small molecules activate sentinel cells residing in muscle tissue (resident cells), such as mast cells, which triggers sterile inflammatory responses (19). In addition to anaphylatoxins, it is now known that tissue damage also liberates intracellular proteins and molecules normally sequestered in the extracellular matrix, which activate resident cells once they are released at the site of the injury (20). In other words, the mechanical stress induced by a traumatic muscle injury releases a wide variety of factors that activate different resident cell types to initiate the inflammatory process(21). In addition to their role in the orchestration of the inflammatory process, the factors released by mast cells also directly stimulate the proliferation of satellite cells (22,23). These findings indicate that inflammatory cells coordinate the muscle healing process right from the start.

Development of the inflammatory process: One of the major roles of the early mediators of inflammation released by resident cells is to increase vasodilatation, vascular permeability and the expression of adhesion molecules to allow the infiltration of inflammatory cells into peripheral tissues from the blood circulation (24). The first cell type to migrate into the injured tissue from the blood are neutrophils, followed by blood monocytes which are converted into macrophages when reaching muscle tissues (25). Neutrophils are able to phagocytose cell debris in order to clean the injured zone; however, they also release proteolytic enzymes and reactive oxygen species that can induce induce secondary damage to the intact tissue near the injured zone (16,26). This detrimental side effect is dependent on the type and the intensity of the muscle injury; in other words, a severe muscle injury leads to more important neutrophil-induced collateral damage compared to a mild injury (27). During this inflammatory phase, which typically lasts 72 hours, both neutrophils and

167 macrophages, along with other resident cell types (*e.g.* endothelial cells, fibroblasts,
168 myofibers and satellite cells), release cytokines (*i.e.* secreted proteins important for cell
169 signalling) that stimulate inflammatory cell recruitment (16).

170 *Resolution of the inflammatory process*: Contrary to the original belief that the
171 dampening of the inflammatory response is a passive process caused by the arrest of pro-
172 inflammatory factor secretion, recent discoveries showed that resolution of inflammation is
173 an active step that involves complex cellular and molecular interactions (28). At the cellular
174 level, it was shown that macrophages switch from a pro-inflammatory phenotype (M1
175 macrophages) to an anti-inflammatory phenotype (M2 macrophages) approximately 2 days
176 after a muscle injury (29). Additionally, these subsets of macrophages have very different
177 functions. M1 macrophages phagocytose muscle cell debris and release pro-inflammatory
178 factors that stimulate myoblast proliferation. On the other hand, M2 macrophages release
179 anti-inflammatory molecules and growth factors that stop myoblast proliferation and
180 stimulate their differentiation, fusion, and myofiber growth. Therefore, the switch in
181 macrophage phenotype is essential to a timely coordination of the activity of myogenic cells.
182 A similar switch was also observed at the molecular level during muscle regeneration, where
183 the biosynthesis of proinflammatory lipid mediators is progressively replaced by anti-
184 inflammatory and pro-resolving lipid mediators. This programmed class-switching of lipid
185 mediators supports the idea that the resolution of inflammation is predetermined from the
186 beginning of the inflammatory response (28,30,31). Particularly, the enzyme cyclooxygenase
187 (COX)-2 is directly implicated in this lipid mediator class-switching. Both proinflammatory
188 and anti-inflammatory molecules are generated by COX-2 at different stages of the
189 inflammatory process (32,33), which is particularly important because COX-2 is the enzyme
190 targeted by NSAIDs. Since physiotherapists are frequently treating patients consuming
191 NSAIDs, they should be aware that COX-2 inhibition does not only blunt the pro-
192 inflammatory response but also inhibit the resolution of inflammation, which has a direct
193 effect on muscle healing.

Overall, muscle regeneration is intimately related to the different phases of inflammation in the context of acute injury (Figure 2: upper panel). Coordinated inflammatory cell recruitment orchestrates satellite cell activity and ensures optimal muscle recovery. Because of the importance of physiotherapy in the optimization of the repair phase, the relationship between inflammation and muscle regeneration will be further discussed in the following section.

The impact of NSAIDs on acute muscle healing

The enthusiasm for the use of anti-inflammatory drugs in order to control the inflammatory process has pushed many research groups to study the impact of partial or complete inhibition of inflammation on muscle repair. Inhibition of COX-2 with specific inhibitors or using COX-2-deficient animal models demonstrated that blocking this pathway diminishes proliferation, differentiation and fusion of satellite cells, and results in impaired skeletal muscle growth, delayed skeletal muscle repair and increased fibrosis (34,35). In human, administration of NSAIDs failed to improve the efficacy of physiotherapy treatment following acute hamstring injury (36). Moreover, using a model of maximal eccentric contractions-induced muscle injury in humans, Mikkelsen *et al.* have demonstrated that local injections of indomethacin, a nonspecific COX inhibitor, by microdialysis catheters into the vastus lateralis muscle for 7.5 hours during the exercise day suppressed the exercise-induced increase of satellite cells at day 8 post-exercise (37). Mackey *et al.* reached the same conclusion by studying muscle biopsies of healthy male endurance athletes that received 100 mg of indomethacin every day from 4 days before a 36-km run-induced muscle injury until day 8 post-run (38). In another study, the same first author showed that ibuprofen-treated young men have higher satellite cell content 7 days after an electrical stimulation-induced muscle injury compared to the placebo group. These results suggest that there are differences in satellite response to NSAIDs depending on the type of injury.

Muscle protein synthesis is also affected by NSAID consumption. In a murine model of chronically overloaded plantaris muscle (induced by the surgical removal of the

gastrocnemius and soleus), the administration of nonspecific COX inhibitor ibuprofen in the drinking water of rats inhibited plantaris hypertrophy by 50% following 14 days of overloading (39). Similar results were also observed in mice (40). In contrast, using an experimental procedure on healthy elderly patients in which one lower limb was immobilized in a cast during 2 weeks followed by 6 weeks of retraining, Dideriksen *et al* showed that NSAID consumption (ibuprofen 1,200 mg/day) did not affect muscle mass and strength (41). However, their NSAID treatment did not significantly affect the circulating levels of inflammatory markers. Therefore, because of differences in the type of injury, its severity, and the efficacy of the anti-inflammatory modality used across studies, some discrepancies exist on the effect of NSAIDs on muscle regeneration. Nonetheless, there is accumulating evidence indicating that the dampening of the inflammatory process during acute injuries leads to impaired muscle growth and regeneration in animals and humans.

Different animal models were used to analyze the specific impact of each individual inflammatory cell type during muscle healing. For instance, by specifically depleting neutrophils, it has been shown that even if these cells are known to induce secondary damage during the inflammatory process, they also contribute to muscle growth and repair by cleaning cell debris and activating satellite cells (42). Moreover, injured muscles of mice depleted of neutrophils present larger areas of necrotic tissue than control mice at 7 days post-injury (43). Other inflammatory cells, the monocytes/macrophages (monocytes circulate in blood and become macrophages once they migrated in the tissue) have been much more extensively studied in the literature for their role on muscle repair. Many studies have demonstrated that impaired macrophage accumulation leads to defective skeletal muscle healing. For example, by depleting blood monocytes, Summan *et al.* have established that the decrease in macrophage accumulation in the injured muscle was accompanied with persistent necrotic myofibers and increased fat accumulation into muscle at days 9 and 14 post-injury, respectively (44). Accordingly, Arnold *et al.* (2007) showed that the depletion of blood monocyte at the time of injury entirely prevented muscle regeneration whereas the

depletion of intramuscular macrophages from day 5 post-injury caused a reduction in myofiber diameter (29). Similar conclusions have been reported by many other groups using various models (45,46). Taken together, these results clearly indicate that (1) the various phases of the inflammatory process play a critical role in orchestrating muscle regeneration following an acute injury and (2) pharmacological inhibition of the inflammatory process impairs acute muscle healing.

To optimize physical therapy treatments, it is essential to associate the patient's clinical symptoms to the ongoing muscular healing phase. Therefore, physiotherapists must understand inflammation on a fundamental level to determine whether anti-inflammatory modalities will delay or optimize muscle repair. Notably, anti-inflammatory properties of a treatment are commonly confounded with its analgesic effect. For instance, cryotherapy has a null to mild effect on pro-inflammatory markers following exercise-induced muscle damage (47). Moreover, the administration of ibuprofen (1,200 mg per day) did not significantly reduced the infiltration of neutrophils or macrophages following exercise-induced muscle damage (48,49). Thus, it is important to keep in mind that many therapeutic modalities have a limited effect on inflammation and act mostly on pain reduction. Since pain is a frequently reported symptom, the analgesic versus anti-inflammatory effect of a given modality must be fully understood by physiotherapists to determine the best therapeutic approach in order to promote patient recovery.

The chronic inflammatory process

Skeletal muscle has an impressive ability to respond to its local or systemic environment. This plasticity is essential for skeletal muscle adaptation to exercise or growth stimuli, but it can be deleterious in the context of chronic inflammation. The persistence of pro-inflammatory signals affects the regenerative capacity of satellite cells and consequently impairs skeletal muscle healing leading to inappropriate repair mechanisms, such as muscle fibrosis and fat accumulation (50,51). These unresolved inflammatory events can originate

from local perturbations (e.g. repetitive muscle traumas, muscle dystrophies) or systemic disorders (e.g. cancer, chronic obstructive pulmonary disease (COPD), rheumatoid arthritis) (52,53). In contrast to the literature on acute inflammation, the overall quality of evidence on the influence of chronic inflammation on muscle repair is relatively weak. Here, we discuss well-supported studies in order to understand the physiological impact of local or systemic factors-induced chronic inflammation on skeletal muscle healing.

Systemic factors: Different groups have conducted elegant studies to document the effect of the systemic environment on the muscle repair process (54,55). Particularly, it was shown that aging is related to a higher concentration of pro-inflammatory systemic factors **(also called "inflammaging"),** which impairs the resolution of the inflammatory process and contributes to different diseases (56). This chronic up-regulation of pro-inflammatory factor levels impairs macrophage polarization shift from M1 toward M2 (57,58). Using different animal experiments, it was demonstrated that the rise in the expression of systemic pro-inflammatory factors during aging directly impairs skeletal muscle healing. For example, an allograft experiments between young and old rats showed that a muscle collected from an old rat and transplanted into a young animal has improved muscle regeneration compared to a muscle collected from a young rat and transplanted into an old rat (55). Likewise, connecting the blood circulatory system of an old mouse to that of a young mouse improves satellite cell function in the old mouse (54). Altogether, these results indicate that systemic factors directly influence the cells involved in skeletal muscle healing and growth.

In addition to slowing down the repair process, there is evidence indicating that this chronic low-grade inflammatory state is associated with a reduction in the synthesis and an up-regulation in the degradation of contractile proteins in muscle fibers (59). Systemic pro-inflammatory factors produced in conditions such as COPD (60), chronic kidney disease (61) and aging (62,63) were demonstrated to be associated with muscle wasting. Some of the systemic factors affecting skeletal muscle healing and growth have been identified. High levels of tumor necrosis factor-alpha (TNF-alpha), were detected in the serum of patients

with COPD and were correlated with impaired myoblast differentiation and muscle wasting (also known as cachexia) (64). Similarly, high levels of the pro-inflammatory cytokine interleukin-6 (IL-6) were observed in the serum of patients suffering from different pathologies such as prostate cancer (65) and also in the serum of healthy patients during aging (66,67). Notably, IL-6 was shown to impair satellite cell function (68) and promote muscle wasting (69). Overall, these studies indicate that many chronic disorders generate systemic pro-inflammatory factors that impair muscle healing.

Local factors: As discussed previously, muscle healing is a tightly coordinated process, which is regulated by various molecular and cellular components that evolve during the different healing phases (Fig 2, upper panel) (70). However, a second injury to an already regenerating muscle can desynchronize this healing process (29,71). To study the effect of asynchronous muscle regeneration, Dadgar *et al.* used a mouse model in which muscles were injured with an intramuscular injection of notexin (toxin from snake venom) and then followed by a second injection 4 or 10 days later (repetitive local injuries) (71). Successive muscle injuries separated by 4 days led to a prolonged and persistent inflammatory response, while muscle injuries separated by 10 days caused an exaggerated production of the pro-fibrotic factor TGF-beta (transforming growth factor-beta), which led to muscle fibrosis (71). Similarly, another study showed that repetitive muscle injuries (intramuscular toxin injection 3 times every 5 days) lead to the exhaustion of the number of satellite cells (72). Altogether, these results showed that local interferences of the myogenic process strongly impair muscle healing.

A similar pattern of chronic muscle injuries is observed in Duchenne muscular dystrophy (DMD), a severe genetic disease characterized by a mutation in the gene that encodes for the structural protein dystrophin. In absence of dystrophin, the muscle fibers are fragile and prone to injury, which leads to repetitive cycles of degeneration and regeneration. Muscle biopsies from patients suffering of DMD have shown that inflammatory cells and pro-inflammatory molecules are highly expressed at various stages of the disease (73).

Consequently, macrophages are chronically present in the muscle, where they fail to switch to the anti-inflammatory phenotype (M2 macrophages) and rather adopt an hybrid phenotype that produces large quantity of TGF-beta, thereby stimulating fibrosis (74,75). The rapid and overwhelming synthesis of extracellular matrix results in the formation of scar tissue that ultimately prevents complete muscle healing (Figure 2: lower panel). Furthermore, persistent inflammatory cell activity causes the release of numerous pro-cachexia factors such as TNF-alpha that stimulate muscle wasting (76). Inflammatory cells also release enzymes and oxidative factors that lead to cell membrane leakage and cause additional collateral damage to the muscle. In summary, the perturbation of muscle repair by repetitive local injuries contributes to an excessive and/or persistent inflammatory process and subsequently to the progression of the disease and muscle dysfunction.

The impact of anti-inflammatory drugs on muscle healing in chronic inflammatory conditions.

Medical management of patients with chronic inflammatory conditions is a challenging clinical problem faced by health professionals. Treatment plans usually include long-term NSAID or steroidal anti-inflammatory drugs (SAID) administration. Contrary to what is observed in acute muscle injuries, the use of anti-inflammatory drugs may have potential beneficial effects on some chronic muscle disorders. For instance, the administration of prednisone following asynchronous injuries, induced by delayed injections of notexin, was shown to blunt the chronic inflammatory condition, which diminished the production of TGF-beta and reduced muscle fibrosis (71). Thus, SAID administration restored the balance in the inflammatory process and improved muscle regeneration. Similar observations were also made in dystrophic muscles, which are subjected to an uncontrolled inflammatory process. SAID administration reduced muscle damage in the diaphragm of dystrophin-deficient *mdx* mice (mouse model of Duchenne muscular dystrophy) (77), and increased grip strength, motor coordination and maximum force of extensor digitorum longus (EDL) muscles (78). Similarly, SAID treatment to young boys suffering from DMD prolongs

mobility, improves cardiac and pulmonary function and delays the need for assistance with feeding (79). However, both in mice and humans, the positive effects of SAIDs are limited in time and are progressively lost after a few months to years (78,80). Part of this time-limited effect might be caused by the fact that while SAIDs are very efficient at downregulating inflammatory activity, their prolonged use can have harmful side effects. For instance, glucocorticoids activate cellular signalling pathways involved in protein degradation, which promotes muscle atrophy (81). Moreover, SAIDs reduce the proliferation and differentiation of myoblasts (82). Thus, while the dampening of the chronic inflammatory process is responsible for the short-term beneficial effect of SAIDs on muscle healing in DMD, the long-term administration of these pharmacological agents potentially contributes to muscle wasting.

The efficiency of anti-inflammatory drugs on muscle healing also depends on the origin of the inflammatory process, i.e. local or systemic. For example, COPD is usually associated with systemic inflammation, which correlates with muscle fiber type changes, muscle atrophy, and impaired muscle regeneration. Several randomised controlled trials studied the impact of anti-inflammatory therapies on the muscles of patient suffering from COPD; however all have failed to show significant improvement in muscle function (83). Similarly, it was shown that NSAID administration to aged patients with low-grade chronic inflammation is inefficient in decreasing systemic inflammation and does not affect muscle response to exercise (84). Furthermore, NSAID administration to patients suffering from osteoarthritis (which is correlated with a low-grade systemic inflammation (85)) did not affect exercise-induced response (86). Therefore, NSAIDs are usually inefficient to treat conditions associated with a systemic inflammatory response.

In summary, anti-inflammatory drugs could have beneficial and adverse effects on muscle regeneration depending on the chronic inflammatory state (local or systemic) and on the type of injury or disease. Thus, anti-inflammatory modalities could be part of a therapeutic

strategy along with physical therapy in order to treat chronic disorders, but their use should be carefully selected based on scientific evidence.

Therapeutic relevance

In this manuscript we discussed fundamental and clinical articles to shed light on the current state of the literature regarding the effect of inflammation and anti-inflammatory modalities on muscle healing. As a result of our investigation, we propose a concept map for the use of anti-inflammatory modalities in a clinical setting (Figure 3). First, one should determine whether the condition involves an inflammatory response. Indeed, there is a common misconception among clinicians that chronic pain is always associated with chronic inflammation. However, many painful conditions such as tendinopathies are not clearly associated with inflammatory cell infiltration. Therefore, treating these patients with anti-inflammatory modalities is primarily based on patient comfort assessment without considering the physiological impact of NSAID intake on tissue healing and the evidence-based use of anti-inflammatory modalities. If the condition is associated with an inflammatory response, the clinician should determine whether it is acute or chronic. As discussed, a controlled and coordinated inflammatory process is beneficial for muscle healing. If the inflammation is considered chronic or excessive, the clinician should determine whether this inflammatory response is local or systemic. As demonstrated previously, anti-inflammatory modalities are usually effective to dampen local inflammation but not systemic inflammation. Thereafter, the appropriate anti-inflammatory treatment should be determined. The optimal modality should dampen the inflammatory process (which is not the case for many approaches whose effects are mostly analgesic) and its use should have been validated by clinical studies for that specific condition. Finally, clinicians must keep in mind that the prolonged use of systemic anti-inflammatory modalities such as SAIDs might have detrimental side effects on skeletal muscle by promoting muscle wasting.

Perspectives

Overall, inflammation and muscle regeneration are closely interconnected through complex cellular, physical and chemical interactions. In acute conditions, these interactions are beneficial for muscle healing; however, they can also be detrimental in chronic or excessive inflammatory conditions. Therefore, the therapeutic plan of physiotherapists must take into account the delicate balance between the reduction of the excessive inflammatory state and muscle regeneration (Figure 3). As the elderly population grows, physiotherapists will increasingly be confronted to complex local and systemic inflammatory conditions. Therefore, there is a need for professional updates on evidence-based use of anti-inflammatory modalities (5). This perspective paper was intended to build a bridge between fundamental research and clinical use of anti-inflammatory modalities. In a few years, we will be able to evaluate the effect of NSAID prescription on the clinical practice of UK physiotherapists. Therefore, additional studies will be needed to further characterize the effect of anti-inflammatory modalities on musculoskeletal healing to ensure that fundamental evidence translates into effective clinical practices in physiotherapy.

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Figure legends

Figure 1: The activity of satellite cells during muscle regeneration

Following an injury (day 0), resting satellite cells (adult muscle stem cells) are activated and start proliferating (day 1). The proliferating cells (myoblasts) reach their peak approximately 3 days after the injury, when they will stop proliferating and start to differentiate. Differentiated myoblasts will fuse into myotubes (immature myofibers) around day 4 to 7 after the injury. Newly formed myofibers will grow into mature myofibers over the next few weeks. Satellite cells will return to their resting state, but will be poised for a future injury. The satellite cells are guided through these different phases by the activity of inflammatory cells (see Figure 2).

Figure 2: Effect of acute and chronic inflammatory processes on muscle regeneration.

In the context of acute inflammation (upper panel), neutrophils and pro-inflammatory macrophages (M1) massively accumulate in the injured muscle. The M1 macrophages release a variety of pro-inflammatory agents such as TNF-α (tumor necrosis factor-alpha) that will foster the activation and proliferation of satellite cells. Thereafter, during the phase of resolution of the inflammatory process, the M1 macrophages switch to an anti-inflammatory phenotype (M2). M2 macrophages stimulate the differentiation of myoblasts in myotubes and promote the growth of muscle fibers. In chronic inflammation (lower panel), the persistence of neutrophils impairs macrophage conversion from M1 to M2 profile and these cells adopt a hybrid phenotype, which impairs muscle healing and triggers fibrosis by releasing an exaggerate amount of TGF-beta.

Figure 3: Concept map for the use of anti-inflammatory modalities

This decision-making tree shows the general guidelines for the evidence-based use of anti-inflammatory modalities for the treatment of muscle disorders in a clinical setting.

Figure 1

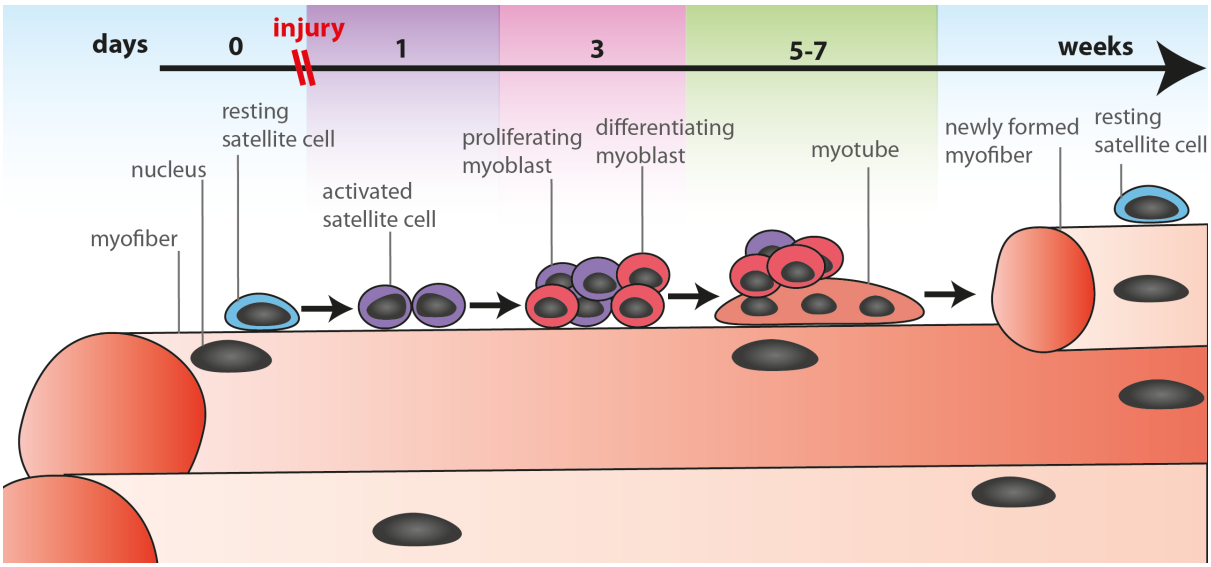
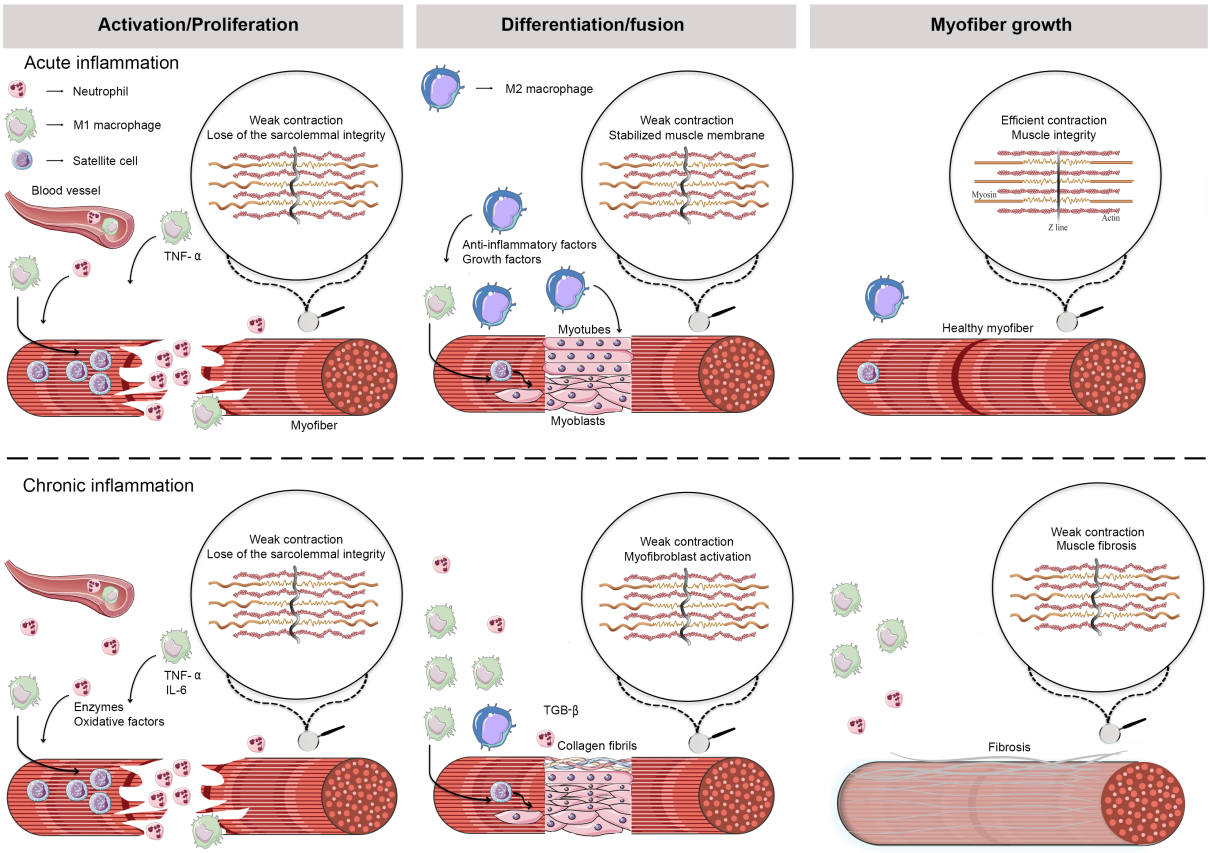

days
0
injury
1
3
5-7
weeks
resting satellite cell
nucleus
myofiber
activated satellite cell
proliferating myoblast
differentiating myoblast
myotube
newly formed myofiber
resting satellite cell

Figure 2


Activation/Proliferation
Differentiation/fusion
Myofiber growth
Acute inflammation
Neutrophil
M1 macrophage
Satellite cell
Blood vessel
TNF- α
Weak contraction
Lose of the sarcolemmal integrity
Myofiber
M2 macrophage
Weak contraction
Stabilized muscle membrane
Anti-inflammatory factors
Growth factors
Myotubes
Myoblasts
Efficient contraction
Muscle integrity
Myosin
Actin
Z line
Healthy myofiber
Chronic inflammation
TNF- α
IL-6
Enzymes
Oxidative factors
Weak contraction
Lose of the sarcolemmal integrity
Weak contraction
Myofibroblast activation
TGB-β
Collagen fibrils
Weak contraction
Muscle fibrosis
Fibrosis

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735 **Figure 3**

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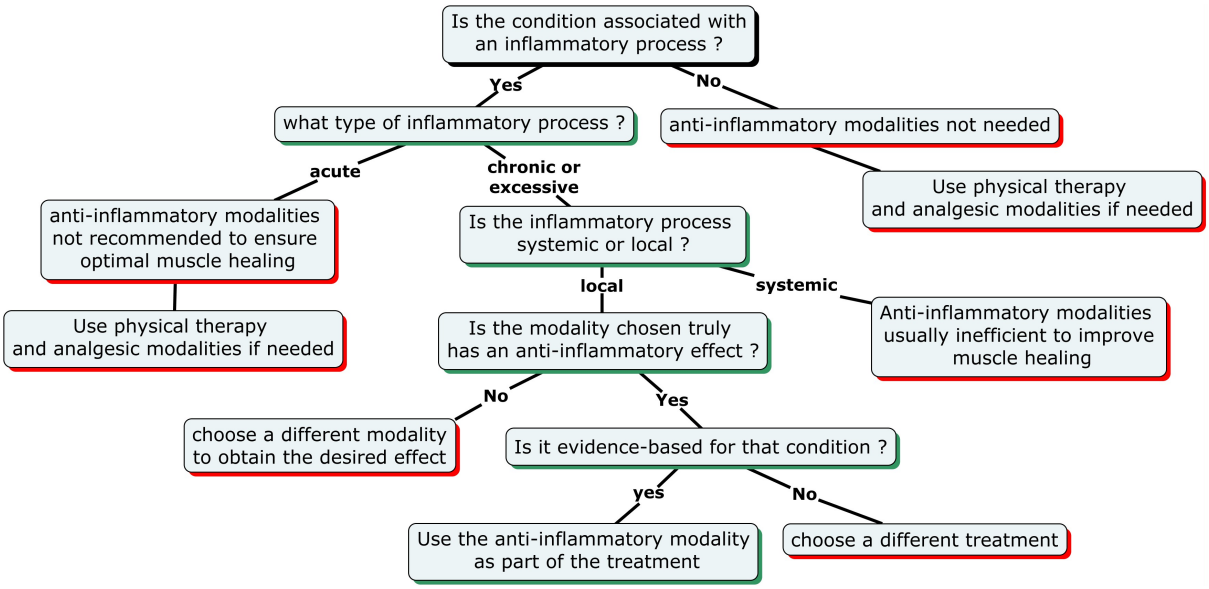

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741 **Table 1:** Brief description of the different cell types discussed in this manuscript

742

Cell types	Description
Satellite cells	Adult muscle stem cells responsible for muscle healing. Satellite cells are inactive in a resting muscle.
Myoblasts	Activated satellite cells that proliferate and differentiate during muscle regeneration.
Myotubes	**Multinucleated cells formed by the fusion of myoblasts. Immature myofibers**
Myofibers	Multinucleated cells that contains the contractile proteins responsible for muscle contraction.
Mast cells	Inflammatory cells residing in different tissues. Once activated they play a key role in the initiation of the inflammatory process
Neutrophils	Inflammatory cells that circulate in blood and rapidly infiltrate a tissue after an injury where they play a key role in debris clearance.
Monocytes	Inflammatory cells that circulate in blood and infiltrate the injured tissue where they differentiate into macrophages.
Macrophages	Inflammatory cells which can switch from a pro-inflammatory phenotype (M1) to an anti-inflammatory phenotype (M2).

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