

# Redefining the satellite cell as the motor of skeletal muscle regeneration

Benyam Yoseph<sup>1,2</sup> and Shay Soker<sup>2</sup>

<sup>1</sup> Department of Surgery, Wake Forest Baptist Health, Winston-Salem, NC, USA;

<sup>2</sup> Wake Forest Institute for Regenerative Medicine, Winston-Salem, NC, USA.

\* **Corresponding Author:** [ssoker@wakehealth.edu](mailto:ssoker@wakehealth.edu).

**Abstract:** Muscle stem cells, also known as satellite cells, hold a central role in skeletal muscle endogenous regeneration potential. However, injury to the muscle alters satellite cell activation, proliferation, and differentiation, which can lead to muscle damage, fibrosis, and functional disability. Additionally, the injured microenvironment can worsen their survival. The etiology behind scar formation is multifactorial. Upon injury, the muscle undergoes a self-repair process. The regeneration process is comprised of degeneration, inflammation, angiogenesis, neogenesis, myogenesis, and tissue remodeling. Each of these components interacts in a complex ecosystem that is under constant surveillance and is tightly regulated. If the crosstalk among these components is disrupted, the healing process is affected and consequently, scar tissue will form. This review highlights how satellite cells act as the motor for normal muscle regeneration and repair.

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## 1. Introduction to muscle regeneration

The skeletal muscle comprises 40% of the human body and in response to physiological changes and minor injuries/tears, it can remodel, repair, and regenerate itself [1]. However, as we age, this capacity declines and the success of myogenesis depends on the extent of the initial injury [2, 3]. Fibrosis is the result of abnormal healing due to severe injury and aging. Additionally, aging is associated with a decrease in muscle mass [4, 5]. The skeletal muscle regeneration capacity is due to satellite cells (SCs), its progenitor, and stem cells. These cells become activated during injury to reconstruct the damaged myofibers. This unique capacity of SCs makes them the “motor” of skeletal muscle regeneration.

SCs have long been characterized as the motor for muscle regeneration. This has been hypothesized to be due to their ability to self-renew and maintain the SC population, as well as to proliferate and differentiate to form myofibers [6, 7]. A loss of or decrease in the activation of SCs during injury has been associated with abnormal healing and fibrosis formation [8].

Recent dramatic advances in our understanding of how the SCs propagate muscle healing have led to a

novel investigation into how increasing SC activation, proliferation, and differentiation may be targeted for therapeutic gains in skeletal muscle regeneration. This is highly significant, considering musculoskeletal disorders are a primary cause of disabilities in the military and civilian populations.

The estimated total cost associated with musculoskeletal disorder/disabilities exceeds \$849 billion, equal to 7.7 percent of the GDP [9, 10]. Despite this prevalence and decades of advances in medical research, our understanding of the pathophysiology of musculoskeletal disorders is limited such that a paucity of effective therapies exists [11-13]. The purpose of this review is to highlight new insights into the complex balance that exists between SCs and myogenesis, as well as to identify how an injury in this relationship can lead to significant scar tissue formation or even disability (Fig. 1).

## 2. Skeletal muscle development

Muscle regeneration after an injury is similar to muscle development during embryogenesis and it seems to follow the same pathway. Thus, understanding skeletal muscle development would help to understand the events during muscle repair.

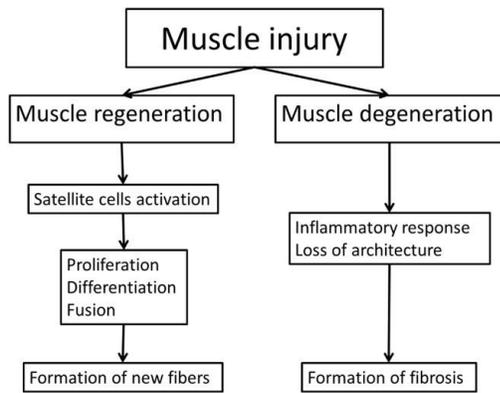


Fig. 1. Normal and abnormal skeletal muscle regeneration. During a minor injury/tear, muscle fibers regenerate via the activation, proliferation, and differentiation of satellite cells, which then activate and express myogenic transcription factors. In a severe injury and aging, several factors prevent the activation of satellite cells, favoring the formation of dense scar tissue.

The skeletal muscles are derived from mesodermal precursor cells, which originate from the somites (epithelial spheres of paraxial mesoderm) [14-16].

Although the origin of SCs is unknown, they are positive Pax3 and Pax7 genes. Pax3 and Pax7 are early myogenic markers, are members of the paired domain transcription factors, and are co-expressed in the majority of myotomal cells of the somite during embryogenesis [17, 18]. Pax3 is essential for the somite to migrate to muscle precursor cells and Pax7 is required for SC specification during development (Fig. 2).

During an injury, through an unknown mechanism, SCs receive signals from surrounding tissues, which induce the expression of primary myogenic regulatory factors (MRFs), such as MyoD and Myf5. MyoD promotes SC progression to terminal differentiation, while MyoD promotes SC self-renewal. Both Myf5 and MyoD are essential for SC activation and proliferation [19, 20]. The proliferation phase is followed by the expression of secondary MRFs, myogenin and MRF4, which induce terminal differentiation and fusion to form multinucleated myofiber [21] (Fig. 2).

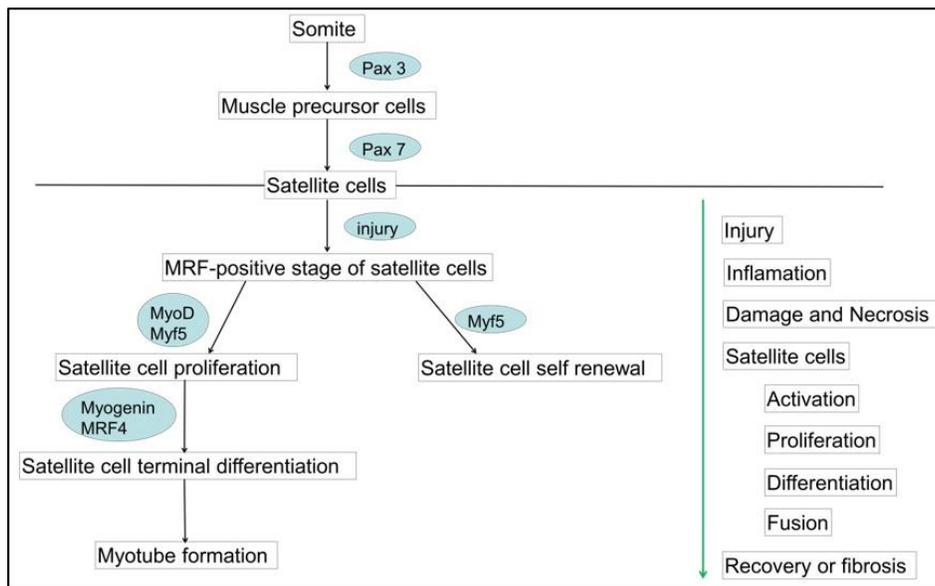


Fig. 2. Satellite cell specification during development and activation, proliferation, and differentiation during a muscle injury.

### 3. Evolution of events in the history of satellite cells

In 1961, Katz and Mauro used electron microscopy to identify an SC, quiescent mononucleated myogenic cells, residing between the plasma membrane of the muscle fibre and basement membrane in the tibialis anticus muscle of the frog [22] (Fig. 3). In 1965, Shafiq

and Gorycki identified the first SCs in mammalian muscle regeneration [23]. In 1968, SCs were noted to undergo mitosis during muscle growth [24]. In 1971, Moss and Leblond found that SCs are able to self-renew [25]. In 1975, SCs were found to generate myoblasts that fuse to form myotubes in vitro [26, 27]. In 1977, Yaffe and Saxel produced a control line of SCs (C2) from the thigh muscles of mice, and it was then recloned

and expanded into the C2C12 cell line by Blau [28, 29]. In 1978, Stichova and Snow after grafting labelled SCs noted donor SCs fuse with host muscle fibers [30-32]. In 1979, 18 years after their discovery, SCs from clonal

cultures were implanted into normal muscle (Fig. 4). For the past three decades, studies on muscle regeneration have been focused on maximizing the fate of grafted SCs.

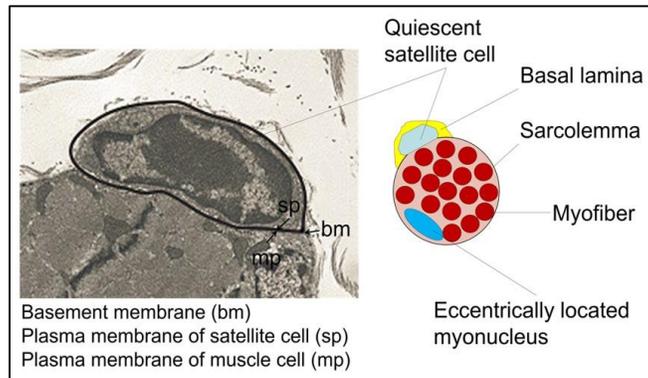


Fig. 3. The electron microscopy of mammalian satellite cell.

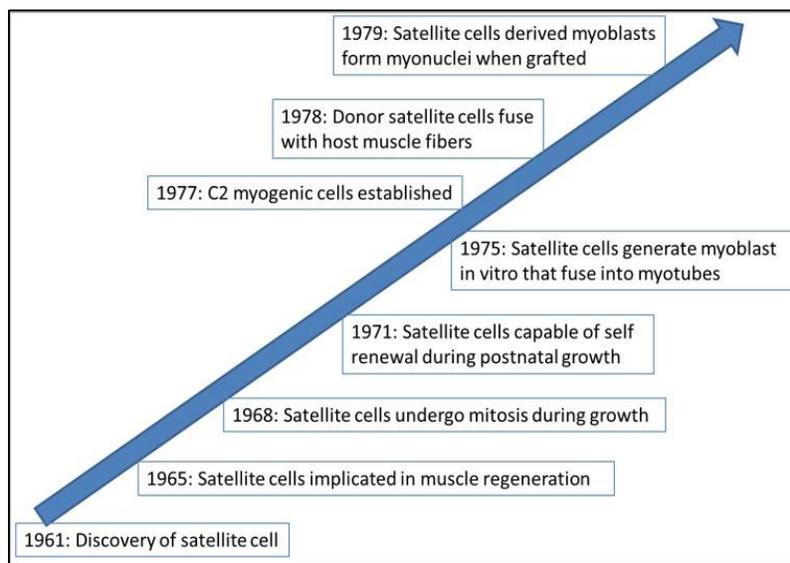


Fig. 4. Timeline of events in the history of satellite cells.

#### 4. The satellite cells as the motor of muscle regeneration during injury

Although the SCs are normally quiescent in adult muscles, they act as a reserve population of cells, are able to self-renew and produce differentiated progeny in response to injury, and contribute to muscle regeneration to replace the lost or damaged muscle [25, 33]. However, severe insults can alter SC activation, proliferation, and differentiation, and this is hypothesized to play a central role in the formation of dense fibrotic lesions, which is a major impairment to

the recovery of muscle function and can lead to muscle contracture and chronic pain, resulting in mobility and decreased quality of life.

The discovery of SCs as the source of myogenic cells needed for myofiber growth, homeostasis, and repair throughout life is important; in addition, understanding the complex relationship that exists between SCs and injury may be used to repair and regenerate damaged or myopathic skeletal muscles or used to act as vectors for gene therapy.

## 5. Factors affecting satellite cell activity

In broad terms, SCs are affected by (i) the immune system, (ii) vasculature, (iii) nervous system, and (iv) the surrounding microenvironment [34, 35]. Each of these components interact in a complex ecosystem that is under constant surveillance and is tightly regulated (Fig. 5).

Cellular events required for skeletal muscle regeneration include inflammation, revascularization, and innervation. The injured muscle undergoes necrosis and inflammation, which is essential to remove the damaged tissue and initiate myogenesis, as well as new blood vessel formation. Reinnervation is also essential for function recovery of the skeletal muscle. The microenvironment (growth factors, cytokines, apoptosis, aging, etc.) can affect SCs' capacity to proliferate, differentiate, and form new muscle. Although different factors are essential for muscle homeostasis and repair, the extent of the injury governs SCs' capacity, resulting in regeneration declines, fibrosis, and impaired function.

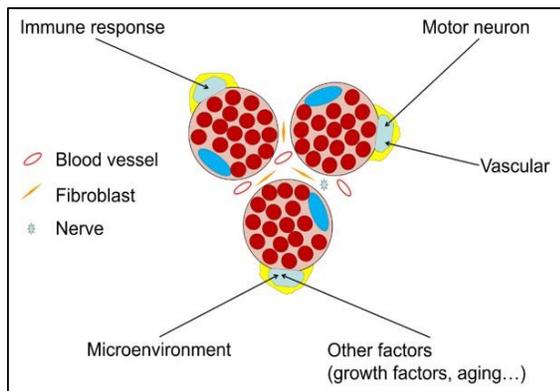


Fig. 5. Multiple factors affect satellite cell activation, proliferation, and differentiation. The immune system, vasculature, nervous system, and the microenvironment work together to prevent muscle damage.

## 6. Functional responses of satellite cells during minor injury

SCs give the skeletal muscle an impressive capacity to remodel, repair, and regenerate itself. The cells undergo physiological changes, such as atrophy or hypertrophy as needed, and they are also able to repair a minor injury, preserving all functional and histological features [36-39].

Skeletal muscle regeneration is a complex phenomenon involving changes to the muscle cells, immune system, vasculature, nervous system, microenvironment, and extracellular matrix. An injury causes muscle fiber destruction and in response to an injury, quiescent SCs become activated, differentiated, and fuse to form myotubes with the support provided by various multiple factors (immune system, vasculature, nervous system, and the microenvironment) either by direct physical contact or by cell signaling [40-43] (Fig. 6).

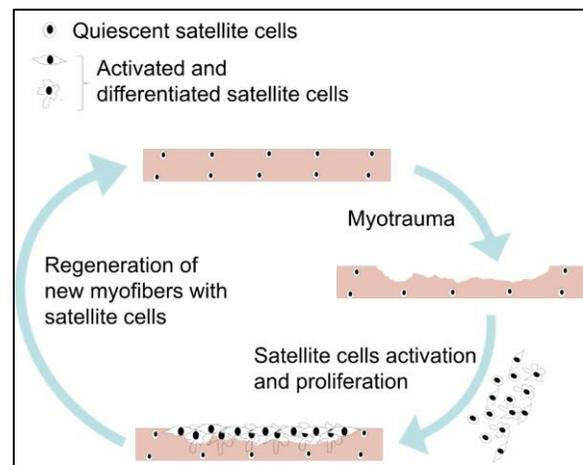


Fig. 6. The schematic representation of the skeletal muscle repair process. During muscle injury, satellite cell activation, proliferation, differentiation, and fusing to form multinucleated myofiber is required to reconstruct the damaged muscle and form new muscle fiber.

## 7. Phases of skeletal muscle regeneration after injury

Studies have shown that the process of regenerating skeletal muscle following an injury is mediated by SCs and it involves three main phases [44-46]. The initial phase is degeneration and an inflammatory response. It starts within minutes after injury and lasts for up to two weeks. The site of the injury is infiltrated by leukocytes, macrophages, and the secretion of pro- and anti-inflammatory cytokines, which can combat infection and clean up the damaged tissue.

The second phase is regeneration; it starts in the first week of injury and consists of SC activation, proliferation, and differentiation, followed by the fusion and formation of mature myofibers.

The last phase is growth and remodeling of the

regenerated muscle. It begins in the second week of injury and involves the upregulation of many growth factors and cytokines to promote angiogenesis to revascularize the newly formed myofibers and neurogenesis to restore function [47-49] (Fig. 7).

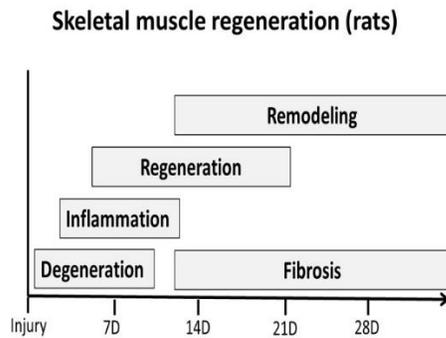


Fig. 7. The different stages of muscle healing after muscle injury in rats. The first event is degeneration and inflammation. The second phase is regeneration, and the final phase is remodeling or fibrosis.

## 8. Aberrant repair and fibrosis development during major injury

Skeletal muscle regeneration during direct (laceration, contusion, and strains) or indirect (ischemia and neurological dysfunction) injuries is a highly orchestrated process and presents an enormous challenge to researchers. Fibrosis is the end result of a cascade of events of abnormal regeneration, characterized by the excessive accumulation of extracellular matrix (ECM) components, such as collagen. Any aberrancy in (i) inflammatory response (acute or chronic); (ii) SC activation, proliferation, and differentiation; or (iii) perturbation to the tissue microenvironment (e.g., growth factor) is associated with impaired regeneration and excessive fibrosis, indicating that a perfect expression of these genes is essential for normal healing [50-54].

Despite the etiology and causative mechanisms, all fibrotic diseases share common cellular and molecular mechanisms. Severe injuries or persistent inflammation disrupts SC activation, favoring their transformation into fibroblast-like phenotypes. The damaged tissue coupled with the persistent disruption and activation of numerous toxic products, such as growth factors, proteolytic enzymes, angiogenic factors, reactive oxygen species, and fibrogenic cytokines, together perturb the tissue microenvironment [55-58]. These

lead to SC dysfunction and mediate the deposition of fibroblast and ECM elements that progressively remodel, destroy, and replace the normal tissue architecture with scar tissue (Fig. 8).

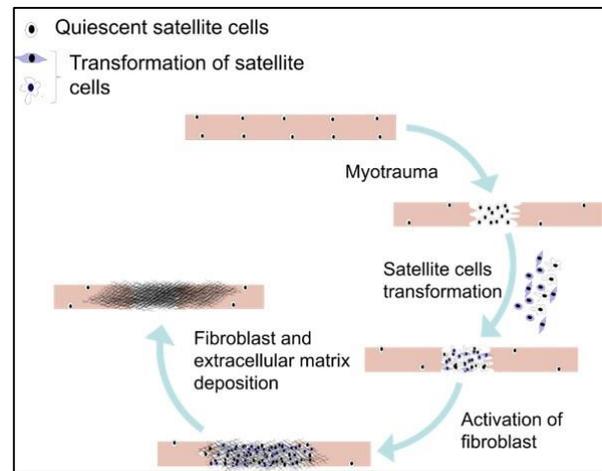


Fig. 8. Schematic representation leading to the activation of fibroblast and ECM deposition. Myotrauma can predispose to inflammation and SCs transformation into fibroblast results in dysfunctional fibrotic tissue.

## 9. Concluding remarks

The concept of SCs as the motor of muscle regeneration is now 60 years old. Tremendous progresses have been made to understand the natural process of SCs in skeletal muscle regeneration. However, the mechanisms that preserve SC homeostasis during injury are not yet fully known. This process is complicated and involves many factors and the interaction with the surrounding microenvironment. SCs are not the solo actors in muscle regeneration during injury. Through a better understanding of the cellular and molecular crosstalk that exists between SCs and the microenvironment, physiological and pathological conditions are necessary for the clinical treatment of tissue repair.

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