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Mathematical Biosciences 333 (2021) 108543

Contents lists available at ScienceDirect

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Original Research Article

A simple model of immune and muscle cell crosstalk during muscle regeneration

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ARTICLE INFO

Keywords:
Muscle
Regeneration
Mathematical modeling
Myotubes

ABSTRACT

Muscle injury during aging predisposes skeletal muscles to increased damage due to reduced regenerative capacity. Some of the common causes of muscle injury are strains, while other causes are more complex muscle myopathies and other illnesses, and even excessive exercise can lead to muscle damage. We develop a new mathematical model based on ordinary differential equations of muscle regeneration. It includes the interactions between the immune system, healthy and damaged myonuclei as well as satellite cells. Our new mathematical model expands beyond previous ones by accounting for 21 specific parameters, including those parameters that deal with the interactions between the damaged and dead myonuclei, the immune system, and the satellite cells. An important assumption of our model is the replacement of only damaged parts of the muscle fibers and the dead myonuclei. We conduce systematic sensitivity analysis to determine which parameters have larger effects on the model and therefore are more influential for the muscle regeneration process. We propose additional validation for these parameters. We further demonstrate that these simulations are species-, muscle-, and age-dependent. In addition, the knowledge of these parameters and their interactions, may suggest targeting or selecting these interactions for treatments that accelerate the muscle regeneration process.

1. Introduction

Skeletal muscle damage, it may be acute or chronic [1], whether serious requiring medical attention or mild, happens very frequently. Muscle injuries may be due to sport contusions and strains, illnesses and diseases such as muscle myopathies, or simply due to muscle overuse [2].

During normal aging, muscle damage can occur more frequently because of intrinsic problems associate to skeletal muscles such as muscle atrophy and muscle weakness [3], and extrinsic factors such as reduced levels of physical activity. This combination of intrinsic and extrinsic factors could lead to increased damage susceptibility and delayed muscle regeneration [4], obviously a combination that creates a positive feedback loop that furthers physical inactivity, and primes the organism for morbidity [5].

It is therefore important to understand the processes involved in skeletal muscle regeneration during development and aging, why they fail in certain situations, and how they can be accelerated to improve recovery in pathological conditions, and also during normal aging. Mathematical models have a major advantage as they can be quickly applied and validated. These models can also be useful to test pharmacological interventions.

Another very important aspect of muscle regeneration is the essential role of the immune system in muscle recovery. Therefore, modeling should account for immune cells and their secreted factors' regulation of new myoblasts [6–8].

In very mild injuries, such as delayed onset muscle soreness (DOMS) usually due to over-exercising or unaccustomed exercise such as running down-hill, there is some inflammatory reaction and activated satellite cells but these cells might not differentiate into myoblasts or this process is rather limited in scope [9]. In severe injuries the myofibers and the connective tissue rupture. Many injuries are moderate in which the sarcolemma ruptures and some myonuclei perish. Three steps have been identified in the healing of a moderate injury, [9] and references therein: (1) Destructive phase in which the myofiber is necrotized a short distance around the damage and an hematoma forms to close the gap; (2) Repair phase in which the immune system

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https://doi.org/10.1016/j.mbs.2021.108543
Received 12 August 2020; Received in revised form 12 January 2021; Accepted 12 January 2021
Available online 16 January 2021
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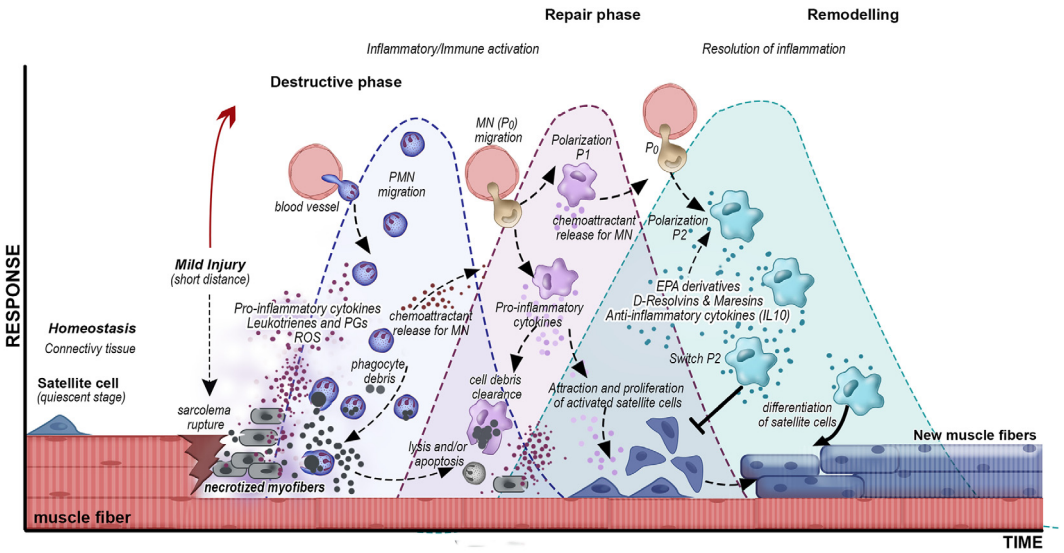

Fig. 1. Schematic illustration of the muscle response to injury and the phases of muscle regeneration/repair. At the lower part of the figure in dark pink color is the muscle fiber (myofiber). In this example, as indicated by the black and red arrows, a mild to moderate injury occurs, which leads to muscle cell damage resulting in sarcolemma rupture, triggering the inflammatory/immune activation in the destructive phase of the response. This initial phase is highly dominated by pro-inflammatory molecules, such as cytokines, reactive oxygen species (ROS), and lipid signaling mediators (LMs, i.e. leukotrienes and prostaglandins [PGs]). Peripheral Mononuclear Monocytes (PMNs) are the first cells attracted to site of injury and begin phagocytosis of the muscle cell debris. As the destructive phase evolves to the repair phase, the non-polarized (denoted by P) monocytes (MN) migrate from peripheral circulation towards the site of injury and polarize into classically activated macrophages (denoted by P_1) in response to LMs released from PMN. This evolution of the destructive phase to the repair phase is commonly refereed to as the remodeling phase of muscle regeneration. P_1 release contributes to the production of pro-inflammatory cytokines, attraction and proliferation of activated satellite cells, phagocytosis/clearance of residual debris from necrotic tissue and PMNs. In the late stage of the repair phase, the production of anti-inflammatory cytokines (IL-10) and LMs (EPA derivatives, D-Resolvins, and Maresins) regulate the resolution of inflammation and new muscle fibers differentiation. These molecules contribute to the switch from classically to alternatively activated macrophage polarization (denoted by P_2), which ultimately inhibit the proliferation of satellite cells and induce their differentiation into new muscle fibers.

removes the necrotized tissue and activates the satellite cells. The activated satellite cells begin the repair of the breached myofiber; and (3) Remodeling, which occurs due to myogeneic differentiation and the fusion of nascent myoblasts into existing damaged muscle fibers or by the formation of new muscle fibers. In this study, our objective was to investigate the repair phase of a moderate injury.

From previous knowledge, we consider that once an aseptic injury occurs, the injured and dead myonuclei release biologically active molecules that are essential for the entire regeneration process, and these molecules also activate the immune associated cells within the skeletal muscle organ system [1]. Lysis and phagocytosis functions of damaged tissue are regulated by neutrophil and macrophage crosstalk [10]. Neutrophils, monocytes and macrophages are involved in cleaning the dead and damaged cells, and in signaling the satellite cells to start differentiating and rebuilding the damaged muscle fibers. These processes need to be finely tuned for successful muscle regeneration. In Fig. 1, a model of a transient and low degree immune response followed by successful muscle healing is described based on previous studies [1,9,11] and our new model. As represented in Fig. 1, in this present study, we propose a mathematical model that considers neutrophils (PMN) and blood derived monocytes/macrophage as the immune cells involved in the clearance of necrotized fibers, as well the main immune cells orchestrating the production of inflammatory mediators (cytokines and LM) throughout the three steps of repair (destructive, repair and remodeling phase). Importantly, our model considers the two macrophage phenotypes involved in healing, classically activated macrophages (as P_1) which are predominant in the pro-inflammatory phase activation of satellite cells; and alternatively activated macrophages (P_2), which are critical for resolution of acute inflammatory and myofibers differentiation [10]. We hypothesize this sequence based in previous knowledge of the role of cytokines and lipid signaling mediators regulating inflammation and tissue healing [6–8,11–13]. Lipid signaling mediators were included in Fig. 1 as potential mediators involved in this process. While AA-derivatives (e.g. LTB4 and PGs) are mainly involved in PMN influx and P_1 differentiation, the EPA-derivatives (Resolvins E and D-series) and DHA-derivatives (e.g. maresins) are involved in the switch from P_1 to P_2 and tissue regeneration in different models [12]. Additionally, Resolvins E- and D-series are suggested to reduce organ fibrosis, reduce PMN transmigration and regulates apoptosis, reduces ROS and pro-inflammatory cytokines generation. Finally, Fig. 1 illustrates the role of damaged myonuclei and new satellite cells along with the muscle healing area. Damaged myonuclei/necrotized myofibers contribute as initiating signals for triggering the immune inflammatory/LMs reaction [9]. The pro-inflammatory macrophages (P_1) stimulate the activation and proliferation of satellite stem cells through the secretion of mediators (e.g. TNFα and IL-6). On the other hand, the switch from P_1 to P_2 macrophages is suggested as a crucial step to inhibit active satellite cells proliferation and stimulate the process of their differentiation into myofibers [10].

Mathematical models can serve a great purpose of complementing in vitro, ex vivo, animal, and clinical studies. When using modeling, someone can test different hypothesis and possibly verify the efficacy of different treatments by performing experiments in silico. In fact, mathematical models are a simplification of reality but they should simulate the physiological system processes.

There is an extensive literature reviewing experimental results on muscle regeneration after injury [1,9,11], the impact of aging and nutrition in this process [10], the role of lipid signaling mediators [12] and other components of immune system regulating muscle growth and regeneration [6–8], as well discussing new strategies for improving muscle repair [13]. Interestingly, while mathematical models are a useful tool to investigate these processes on skeletal muscle regeneration, these studies are still scarce in the musculoskeletal literature. Deshpande, Grayson and Spector [14] present an ordinary differential equation model to investigate the adipose-derived stem cells myogenesis. Proctor and Goljanek-Whysall [15] used computer simulation models to study the influence of different microRNA's on the regeneration process during aging. Jarah et al. [16] developed a mathematical

model of skeletal muscle disease and immune response in the *mdx* mouse, and they concluded that in this model, the immune system response oscillates throughout the life of the mice, and the damaged fibers did not clear completely. There is clearly a need in the muscle research field for the expansion and the arrival of new mathematical models of muscle regeneration.

In the study, we aimed to develop a new mathematical model that expands substantially the number of parameters from any previous mathematical model previously proposed or tested. We expect that our new model will be a useful tool to guide in vitro or in vivo studies, since immune and muscle cell crosstalk are included as drivers of our model during muscle regeneration.

2. Materials and methods

2.1. Modeling assumptions

We consider that the only immune cells involved in muscle regeneration are neutrophils, monocytes and macrophages, classically and alternatively activated or pro-inflammatory and anti-inflammatory, also called P_1 and P_2 (Fig. 1), myonuclei and dead myonuclei, and satellite cells. We assume that only damaged parts of the muscle fibers and the dead myonuclei need to be replaced. The first recruited cells of the immune systems are Neutrophils after muscle damage and they start phagocytizing the dead and damaged myonuclei. Next monocytes polarize into P_1 and P_2 phenotypes. P_1 macrophages digest the rest of the dead myonuclei and the dead neutrophils, signal the P_2 macrophages when the cleaning is complete and P_2 trigger the satellite cells to differentiate into myoblasts.

These myoblasts fuse together as new myofibers. Communication/cross talk among cells is done through the release of a number of chemical factors (many are signaling lipid mediators, e.g. AA derivatives) and we assume, for simplicity, that the amount of each factor is proportional to the number of cells that produce it. We do not consider explicitly the myofibers since we assume that they are only partially damaged, but consider that there are enough new myoblasts to fuse and fix the damaged myofibers.

More specifically, we make the following modeling assumptions: *Neutrophils* arrive in response to pro-inflammatory cytokines and lipid mediators, released upon damage to tissue and die naturally transforming into inactive, lysed neutrophils.

Neutrophils migrate from the surrounding capillaries into the tissue in response to a concentration gradient in the pro-inflammatory cytokines in the region surrounding the site of injury. Neutrophils are assumed to enter the tissue in active form at a constant rate of migration. Neutrophils normally have a short lifespan, undergoing apoptosis within 6–10 h [17]. *Apoptotic neutrophils* are non-functional, unable to move in response to cytokines and have little phagocytic ability. Lysed neutrophils undergo necrosis at a constant rate [17]. *Monocytes* also arrive in response to pro-inflammatory cytokines, released upon damage to tissue. Monocytes migrate from circulation into the damaged tissue where they differentiate into macrophages [17]. Monocyte migration starts in response to chemokines secreted by neutrophils present at the injury site. Monocyte migration also depends on chemokine, which is secreted by injured muscle fibers [10]. Chemicals are released when lysed neutrophils undergo phagocytosis by monocytes and P_1 macrophages; which stimulate monocytes differentiation to additional P_1 macrophages. As the healing progress, chemical factors stimulate the conversion from P_1 macrophages to P_2 macrophages. P_1 macrophages lyse muscle cells and phagocyte cellular debris. During muscle regeneration, P_1 macrophages attract satellite cells to the injured site [10]. We neglect macrophage proliferation and death, assuming instead that macrophages leave the tissue at constant rates. P_1 macrophages respond to signals from apoptotic neutrophils and engulf them at a constant rate, which prevents the neutrophils from undergoing secondary necrosis (called NETosis) and spilling their toxic contents. P_1 macrophages also act to remove damaged myonuclei [17]. Exposure of macrophages to lysed neutrophils greatly increases IL-10 production by macrophages, and therefore increasing the conversion from P_1 to P_2 macrophages, as exemplified in Fig. 1 [7]. P_2 macrophages stimulate muscle cell differentiation, leading to myofiber formation and repair.

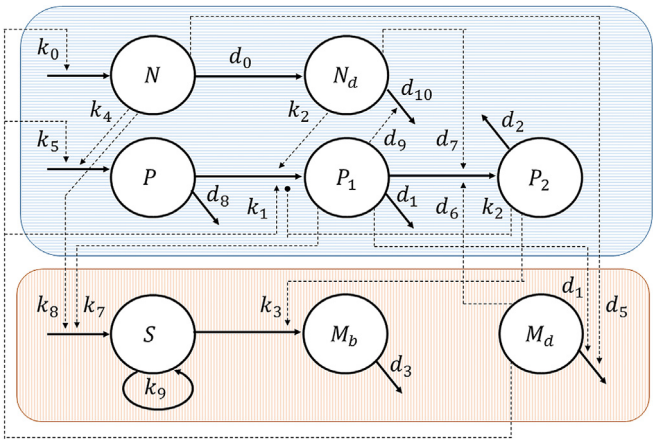

Fig. 2. Flow diagram of the immune (top) and muscle (bottom) cell cross-talk during muscle regeneration.

When regeneration is completed, P_2 macrophages become inactive, and consequently anti-inflammatory cytokine production is interrupted [10]. *Satellite cells* are chemo-attracted to the injury site of the muscle by an increased gradient of cytokines and lipid mediators that are released by neutrophils and P_1 macrophages at the site of tissue damage soon after injury [7,10]. P_2 macrophages stimulate satellite cell differentiation to myoblasts, and their attachment to existing fibers. Satellite cells are quiescent until damage occurs. Excess satellite cells and myoblasts are carried away. When damage is moderate, we assumed that only a few *myonuclei* in each myofiber undergo to necrosis and each of them are replaced by regeneration. Thus, the total number of myofibers is constant. P_2 macrophages stimulate satellite cell differentiation to myoblasts, and subsequent attachment to existing myofibers [10]. Free radical production by neutrophils and P_1 macrophages in injured muscle trigger *tissue debris* for phagocytosis [7]. Neutrophils can also promote the cytolytic capacity of macrophages to lyse muscle cells.

2.2. Model development

We develop a continuous-time ordinary differential system model of the immune and muscle cell cross-talk during muscle regeneration. It is partially based on the skeletal muscle regeneration model in [18], but more detailed and with greater emphasis on the immune system effects on the processes involved. We assume that the cells are homogeneously distributed on the region of study, which is taken to be small enough for this hypothesis to be valid but also large enough to have a large number of cells. The interactions between the different types of cells are presented in Fig. 2, where N, N_d, P, P_1, and P_2 represent the immune cells: active neutrophils, lysed neutrophils, monocytes, classically and alternatively activated macrophages, respectively; while S, M_b, and M_d represent the muscle components: satellite cells, myonuclei, and damaged myonuclei, respectively.

The resulting system of differential equations is as follows:

$$\frac{dN}{dt} = k_0 M_d - d_0 N \tag{2.1}$$

$$\frac{dN_d}{dt} = d_0 N - d_9 P_1 N_d - d_{10} N_d \tag{2.2}$$

$$\frac{dP}{dt} = k_4 N + k_5 M_d - \frac{k_1}{P_2 + k_6} M_d P - \frac{k_2}{P_2 + k_6} N_d P - d_8 P \tag{2.3}$$

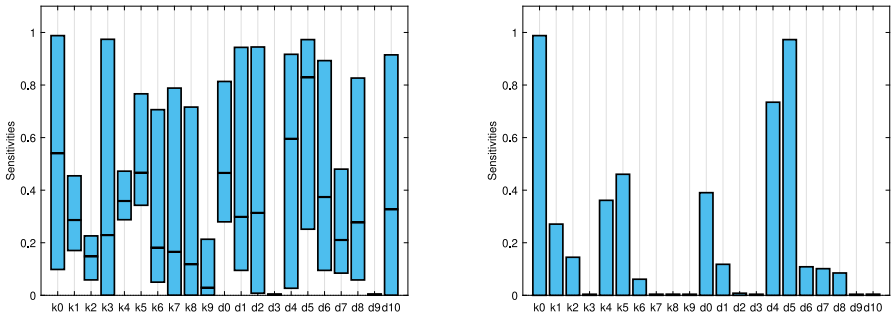

Fig. 3. Maximum, minimum and mean of the indices over all species of the indices averaged over one hundred days (left); Indices for the damaged and dead myonuclei averaged over one hundred days (right).

$$\frac{dP_1}{dt} = \frac{k_1}{P_2+k_6}M_dP + \frac{k_2}{P_2+k_6}N_dP - d_6M_dP_1 - d_7N_dP_1 - d_1P_1 \quad (2.4)$$

$$\frac{dP_2}{dt} = d_6M_dP_1 + d_7N_dP_1 - d_2P_2 \quad (2.5)$$

$$\frac{dS}{dt} = k_9S(S_0 - S) + k_7P_1S + k_8NS - k_3\mathcal{H}(M_d)SP_2 \quad (2.6)$$

$$\frac{dM_b}{dt} = k_3\mathcal{H}(M_d)SP_2 - d_3\mathcal{H}(M_b - M_0) \quad (2.7)$$

$$\frac{dM_d}{dt} = -d_4P_1M_d - d_5NM_d, \quad (2.8)$$

where $\mathcal{H}$ denotes the left-continuous Heaviside step function, and S_0 and M_0 represent the constant resident populations of satellite cells and myonuclei, respectively, in a healthy muscle. We opted for the utilization of the Heaviside function as a practical way of limiting the size of the corresponding populations by making the growth rates zero. Eq. (2.1) states that the time rate of recruitment of neutrophils N is proportional to the number of dead myonuclei M_d and die proportionally to the number of neutrophils. Here, Eq. (2.2) gives the rate of increase of the dead neutrophils N_d is due to this dying neutrophils minus those being eliminated by macrophages P_1 and being carried away. In Eq. (2.3), the rate of increase of the monocytes P is proportional to both the number of dead myonuclei and of dead neutrophils and decreases with the rate of conversion of monocytes to macrophages P_1 given by a Holling type II saturation function and by the rate the monocytes are being carried away. Eq. (2.4) gives the rate of change of macrophages P_1 by the conversion of monocytes and the decrease by conversion to macrophages P_2 and by being carried away. In Eq. (2.5) macrophages P_2 increase from the conversion from macrophages P_1 and decrease by being carried away. In Eq. (2.6) satellite cells S grow logistically and by being signaled by the neutrophils and macrophages P_1 and decrease by being converted to myonuclei to replace the dead ones. Eq. (2.7) states that new myonuclei come from satellite cells and that their number is never greater than the needed for replacement of the dead myonuclei. Finally, Eq. (2.8) gives the rate of elimination of dead myonuclei by both neutrophils and macrophages P_1.

3. Analysis and results

3.1. Sensitivity analysis

Skeletal muscle regeneration depends on diverse interactions between cells and factors. The functions describing these interactions depend on parameters. The proposed model depends on 21 parameters. They have to be determined from experimental data. Some of them like the decay rates can be obtained using the half-life of the species. But parameter estimation is a difficult issue. There are large variations and uncertainties associated with model parameters; therefore, it is important to determine how sensitive the model output is to variations in the parameter values. From estimates of the sensitivity of the model variables to the parameters, it is possible to determine which parameters need to be measured with more accuracy and which are not very relevant to the regeneration process. Also, the information of which parameters have larger sensitivity indices may suggest treatments which will target the interactions that can affect the muscle regeneration the most.

There are different ways of estimating the sensitivity of the cell populations to the different parameters. A local sensitivity analysis determines the model sensitivity to parameter variations over a localized region around given parameter values. But these values are usually not known. A global sensitivity analysis (GSA) investigates the sensitivity over a range of values of the parameters [19–21].

The number of sensitivity indices is large. There are (number of states)x(number of parameters) sensitivity indices which are functions of time. To help deal with this large amount of information, the indices may only be considered at steady solutions, or only at specific times, or only at the average of states over time. In the case of muscle regeneration, the most relevant indices are those that give the influence of the parameters on the dead myonuclei.

Global sensitivity is expensive since it needs to do evaluations over the whole parameter space. A good sampling method is the Latin hypercube [22]. One of the most widely used methods since it is relatively fast is the Fourier amplitude sensitivity test (FAST) based on variance calculations [23,24]. We used IQM tools https://iqmtools.intiquan.com/.

Fig. 3 (left) gives the maximum, minimum and mean of the indices over all species of the indices averaged over one hundred days as calculated using the FAST method. Fig. 3 (right) gives the indices of the dead myonuclei averaged over the same time period. As can be seen from Fig. 3 (right), the largest indices are, k_0, the maximal rate of neutrophil influx due to damaged and dead myonuclei, d_4, the rate at which P_1 macrophages engulf damaged and dead myonuclei, and d_5, the rate at which neutrophils engulf damaged and dead myonuclei.

3.2. Numerical simulations

We perform a set of numerical simulations to demonstrate the performance of the proposed new model. The parameters in the model depend on whether we are working with mice, rats, humans or another species. They also depend on the particular muscle. The parameter values used in the simulations, with their corresponding units, are listed in Table 1. Specific parameter values have been estimated based on available data in [17] and references therein. For parameter values that were not available in the scientific literature, we assigned specific values that gave biologically realistic simulation results and based on the following assumptions about relations between different parameters and processes: the rate of P to P_1 monocyte conversion due to lysed neutrophils is lower than the rate of P to P_1 monocyte conversion due to damaged myonuclei, the maximal rate of monocyte influx due to neutrophils is lower than the maximal rate of neutrophil influx due to damaged myonuclei, the maximal rate of neutrophil influx is greater than the maximal rate of macrophage influx, the rate of satellite cell influx due to classically activated macrophages is greater

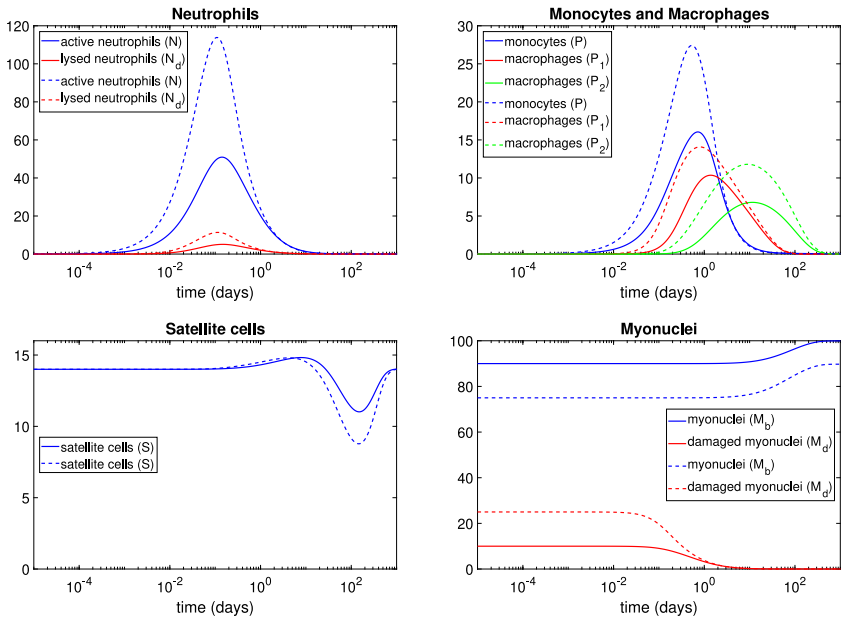

Fig. 4. Log-Linear plots of the immune (top) and muscle (bottom) cells. Simulation results for younger adults with type I muscle, $S_0 = 14$, under muscle damage of 10% (solid lines) and 25% (dashed lines).

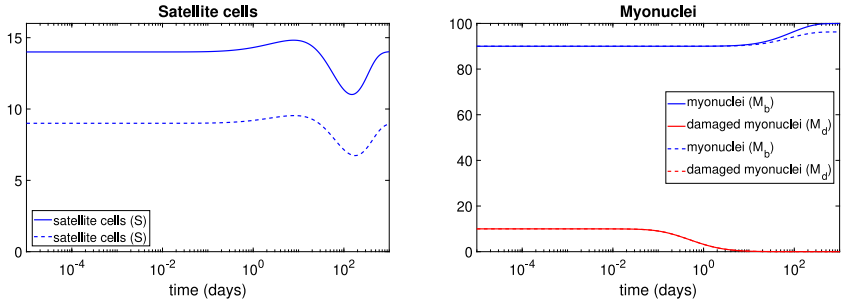

Fig. 5. Log-Linear plots of the muscle cells: satellite cells (left) and myonuclei (right). Simulation results for younger adults with type I muscle, $S_0 = 14$, (solid lines) and older adults with type II muscle, $S_0 = 9$, (dashed lines) under muscle damage of 10%.

than the specific growth rate of satellite cells, the rate of satellite cell influx due to neutrophils is lower than the rate of satellite cell influx due to classically activated macrophages, the rate at which not-needed/extra myonuclei leave the tissue is about the same as the rate at which macrophages leave the tissue, the rate at which neutrophils engulf damaged myonuclei is greater than the rate at which classically activated macrophages engulf damaged myonuclei, and the rate of P_1/P_2 macrophages conversion due to lysed neutrophils is less than the rate of P_1/P_2 macrophages conversion due to damaged myonuclei.

First, we numerically explore the muscle regeneration process in younger adults with type I muscle ($S_0 = 14$) under different severity of muscle damage. As it can be seen in Fig. 4 (bottom-right), the muscle completely recovers in the case of 10% muscle damage (solid lines), while it recovers to less than 90% in the case of 25% muscle damage.

Next, we numerically explore how satellite cells density influences the muscle regeneration process. The number of satellite cells in humans per mm^2 is 14 in younger adults and 12 in older adults for type I muscles; and about 12 in younger and 9 in older adults for type II muscle [25]. We perform a set of numerical simulations, Fig. 5, for younger adults with type I muscle ($S_0 = 14$) and for older adults with type II muscle ($S_0 = 9$) under muscle damage of 10%. As can be seen in Fig. 5 (right), the muscle recovers to only about 96% for older adults with type II muscle (dashed lines), while it fully recovers for younger adults with type I muscle (solid lines).

Finally, we explore how different rates of the fusion index affect the muscle regeneration process after injury and if it is independent of the satellite cell density (Figs. 6 and 7). In our simulations, we model different rates of the fusion index by using different values of the parameter k_3, which represents the differentiation rate of satellite cells to myonuclei.

As we can see in Fig. 6 (right), doubling the value of the differentiation rate constant k_3 in simulations of younger adults with type I muscle leads to an increase in their muscle recovery from below 90% to almost 95%; while doubling the value of the differentiation rate constant k_3 in older adults with type II muscle leads to an increase in their muscle recovery from about 84% to almost 87%, as seen in Fig. 7 (right).

4. Discussion

While there is a reasonable biological understanding of the key steps, genes, and molecules orchestrating the process of myogenesis in development and during muscle regeneration, such knowledge is yet to be matched by robust mathematical models. In this study we have presented a mathematical model of muscle regeneration that takes into account the interactions between the damaged and dead myonuclei, the immune system and the satellite cells. We have performed numerical simulations giving the evolution in time of the different cell populations.

Our new mathematical model expands beyond previous ones by accounting for 21 specific parameters, including those parameters that deal with the interactions between the damaged and dead myonuclei, the immune system, and the satellite cells. We have also used sensitivity

Table 1
Parameters description, values, and units.

	Parameter Description	Value	Units
k_0	Maximal rate of neutrophil influx due to damaged myonuclei	120	1/day
k_1	Rate of P/P_1 conversion due to damaged myonuclei	0.25	1/day
k_2	Rate of P/P_1 conversion due to lysed neutrophils	0.125	1/day
k_3	Differentiation rate of satellite cells to myonuclei	0.001	mm^2/cells/day
k_4	Maximal rate of monocyte influx due to neutrophils	0.6	1/day
k_5	Maximal rate of monocyte influx due to damaged myonuclei	6	1/day
k_6	Saturation constant	1	cells/mm^2
k_7	Rate of satellite cell influx due to P_1 macrophages	0.001	mm^2/cells/day
k_8	Rate of satellite cell influx due to neutrophils	0.0005	mm^2/cells/day
k_9	Specific growth rate of satellite cells	0.0006	mm^2/cells/day
d_0	Rate of neutrophil apoptosis	20	1/day
d_1	Rate at which P_1 macrophages leave the tissue	0.05	1/day
d_2	Rate at which P_2 macrophages leave the tissue	0.01	1/day
d_3	Rate at which not-needed/extra myonuclei leave the tissue	0.04	cells/mm^2/day
d_4	Rate at which P_1 macrophages engulf damaged myonuclei	0.02	mm^2/cells/day
d_5	Rate at which neutrophils engulf damaged myonuclei	0.03	mm^2/cells/day
d_6	Rate of P_1/P_2 conversion due to damaged myonuclei	0.07	mm^2/cells/day
d_7	Rate of P_1/P_2 conversion due to lysed neutrophils	0.014	mm^2/cells/day
d_8	Rate at which monocytes leave the tissue	2	1/day
d_9	Rate at which P_1 macrophages engulf lysed neutrophils	0.01	mm^2/cells/day
d_{10}	Rate of secondary necrosis of neutrophils	200	1/day
M_0	Population size of resident myonuclei	100	cells/mm^2
S_0	Population size of resident satellite cells	9–14	cells/mm^2

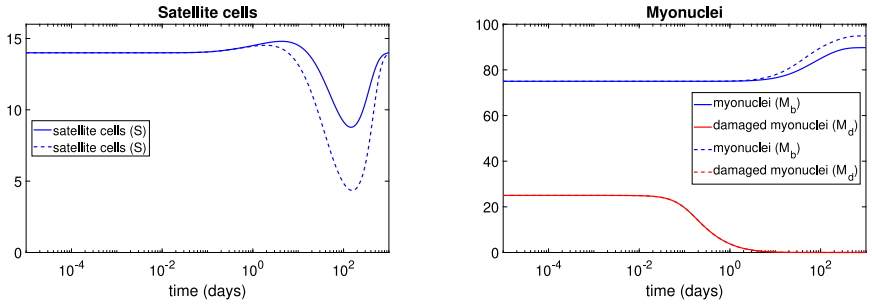

Fig. 6. Log-Linear plots of the muscle cells: satellite cells (left) and myonuclei (right). Simulation results for younger adults with type I muscle, $S_0 = 14$, under muscle damage of 25% for different differentiation rates of satellite cells to myonuclei: $k_3 = 0.001$ (solid lines) and $k_3 = 0.001$ (dashed lines).

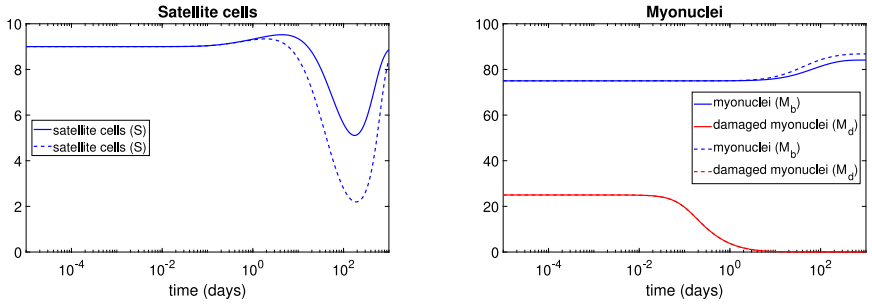

Fig. 7. Log-Linear plots of the muscle cells: satellite cells (left) and myonuclei (right). Simulation results for older adults with type II muscle, $S_0 = 9$, under muscle damage of 25% for different differentiation rates of satellite cells to myonuclei: $k_3 = 0.001$ (solid lines) and $k_3 = 0.001$ (dashed lines).

analysis to determine which parameters have larger sensitivity indices and therefore affect the regeneration process the most, and therefore they have to be determined with more accuracy. In addition, the knowledge of these parameters and the interactions, which they influence, may suggest which interactions should be targeted for treatments that accelerate the regeneration process.

As shown in Fig. 1 and modeling assumptions, neutrophils (N) are involved in the clearance of damage tissue debris, as well they orchestrate the production of inflammatory mediators along with monocytes. Both classically and alternatively activated macrophages (or P_1 and P_2) as well as specific lipid signaling mediators are essential for a normal process of muscle regeneration, and in general to resolve inflammation successfully. It is remarkable that our simulation results, which were validated by our global sensitivity analysis, not only agree, but they support each phase depicted in our Fig. 1. There is clear switch from initially pro-inflammatory molecules (cytokines, leukotrienes, and prostaglandins), into ant-inflammatory (EPA derivatives), to a last phase of resolving molecules (D-Resolvins, Protections, and Maresins). Therefore, the cross-talk between the immune cells and skeletal muscle cells is crucial for any effective regeneration without the formation of fibrosis [12,26,27]. We demonstrated over a series of several studies that Prostaglandin E2 (PGE2) signaling is essential for skeletal muscle cells (mice and humans) myogenesis [28–30]. This hypothesis qualitatively agree with our results.

It is important to emphasize that muscle regeneration is constantly occurring in humans during their lifetime, and in fact the lack or

impairment of such plasticity leads to a number of diseases. Perhaps the only organ system more plastic than skeletal muscles is the skin. Certainly, the highly orchestrated and complex regeneration activity occurs at different rates. Many factors such as age, sex, levels of physical activity, and diet are very likely to influence these rates. We have an extremely poor knowledge of nutrition and we only know the basic macro nutrients, but we do not know the exact compositions of the foodstuff we consume, and potential positive and negative interactions between different food groups.

Importantly, irrespective of such factors, when beyond physiological damages takes place in skeletal muscle, this rate accelerates in the attempt to correct the damage, and under such condition fibrosis might also occur. Thus, we used the model to numerically simulate different practical scenarios. Our computational results, Figs. 4–7, serve the main purpose to illustrate the intrinsic value and power of employing suitable mathematical models even to complex problems such as muscle myogenesis/regeneration. For example, by changing the value of a model parameter in the simulations, it can be predicted how the cell populations change, in particular, how the time to muscle regeneration changes. In this way the efficacy of a proposed new treatment, exercise regime, diet intervention, or drug therapy that modifies certain properties of and/or conditions in the regeneration processes can be easily tested by changing the values of the corresponding modified parameters in the numerical simulations without the required need of biological testing or at least helping to reduce such need.

Thus, the proposed new mathematical model could help determine in more logical and systems biology approach that would also be cheaper and faster, allowing for a larger screening of compounds. For example, in the field of nutritional supplements, instead of focusing only on one single amino acid, there could be thousands of combinations or permutations if primary and amino acid derivatives are added in pairs or trios or in large combinations to test their effects on myogenesis, cellular hypertrophy, etc. The same applies to a project aimed at testing large libraries of thousands of drugs for muscle myopathies. Our models could very quickly provide the best logical and economically viable candidates for biological testing.

It is important to note that based on our numerical simulations we find that the system does not change stability due to parameter variations, demonstrating the robustness of the model. Additionally, satellite cells have a high recovery capacity, activating the necessary ones to converge to the resident population of satellite cells in the damaged area. This fact leads to a successful recovery of muscle regeneration, as summarized in Fig. 1. On the other hand, the scarcity of satellite cells at the time of cell death may cause fibrosis. It is also possible, that signals that accompany these processes such as the amount and nature of lipid signaling mediators and the immune/inflammatory signals could crosstalk with these cells and directly contribute to either resolution of inflammation or fibrosis in a scenario of either balanced/regulated or imbalanced/unregulated inflammatory response.

5. Conclusion

In conclusion, our model offers new insights into the complex process of muscle regeneration and could be used as a potential tool to explore new ways to target acceleration of muscle regeneration under specific conditions. It could be useful in testing specific hypothesis about the reduced regenerative capacity of aged muscles. It is our anticipation that the new model can serve as an initial modeling research stage and it will open new research venues on the process of muscle regeneration/repair.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Fco. Solis was supported by Conacyt, Mexico grant CB-2016 286437. CB and MB are supported by NIH, USA Grants - National Institutes of Aging (NIA) 2-PO1AG039355, NIA-R01AG056504, NIA-R01AG060341. MB is also supported by National Institutes of Neurological Disorders and Stroke, USA (NINDS) 2-R01NS105621; National Institutes of Diabetes, Digestive, and Kidney Diseases Kidney, USA and NIDDK-R01DK119066. The authors are thankful for the generous support from the George W. and Hazel M. Jay and Evanston Research Endowments (MB) and the UTA College of Nursing & Health Innovation Bone–Muscle Research Center (https://www.uta.edu/conhi/research/bmrc/index.php).

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