Journal of Biology and Medicine: Open Access

Short Review| Vol 2 Iss 1

Tissue Engineering Applied to Skeletal Muscle Injuries: An Overview of Therapeutic Perspectives

Ana Luisa Lopes Martins, Arnaldo Rodrigues Santos Jr, and Luciana Pastena Giorno*

Centro de Ciências Naturais e Humanas (CCNH), Universidade Federal do ABC, São Bernardo do Campo, SP, Brasil

***Corresponding author:** Giorno LP, Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, Bloco Delta, sala 204, Alameda da Universidade, s/n, Anchieta, São Bernardo do Campo, 09606-070, SP, Brazil; E-mail: gpluciana35@gmail.com

Received: April 24, 2021; **Accepted:** May 20, 2021; **Published:** May 29, 2021

Abstract

Introduction: ***Skeletal striated muscle has the capacity to convert chemical into mechanical energy, enabling contraction that ensures body movement in the form of muscle work. Muscle deficiencies or injuries compromise the function of this tissue in the body and are classified according to their severity. This condition affects the performance of individuals in activities of daily living, as well as their quality of life. Given the potential of tissue engineering to promote muscle recovery, the aim of this narrative review is to describe techniques that promote skeletal muscle regeneration using spinal muscular atrophy as a model.*** ***Materials and Methods:*** ***Searches were performed in the following databases: United States National Library of Medicine (MEDLINE) via PubMed; Latin American and Caribbean Health Sciences Literature (Lilacs); Cochrane Library; SciVerse Scopus; Web of Science, and Scientific Electronic Library Online (SciELO).*** ***Results:*** ***Using biopolymers and extracellular matrices, tissue engineering attempts to recreate organic tissues capable of regenerating injured tissues or mimicking their activities. Scaffolds produced from animal extracellular matrix, as well as hydrogels and synthetic materials, have been shown to provide support for the growth of new cells in injured tissues.*** ***Conclusion:*** ***Methods developed by tissue engineering aimed at the repair of muscle injuries are important to improve the quality of life of patients. Thus, techniques using bioresorbable materials, such as decellularized biological scaffolds and hydrogels, are promising in the regeneration of muscles, contributing to the recovery of their motor capacity.***

Keywords: ***Muscle regeneration; Bioresorbable polymers; Skeletal muscle; Tissue regeneration; and Spinal muscular atrophy.***

1. Introduction

According to the 2010 Census of the Brazilian Institute of Geography and Statistics (IBGE), 6.96% of the population (13.3 million Brazilians) have some type of motor deficiency. Motor deficiency is a condition of multifactorial etiology characterized by the impairment of the locomotor apparatus, which comprises the osteoarticular, muscular and

Citation: Martins ALL, Santos AR Jr, and Giorno LP. Tissue engineering applied to skeletal muscle injuries: An overview of therapeutic perspectives. J Bio Med Open Access. 2021;2(1):121.

nervous systems. The variable clinical presentation reflects the severity of the involvement of these structures, with loss of function or physical limitations at different levels [1].

Spinal Muscular Atrophy (SMA) and Muscular Dystrophy (MD) (such as Duchenne and Becker) are monogenic neuromuscular diseases and some of the clinical signs result in the atrophy and death of muscle cells. One of the characteristics that differentiate a patient with MD from another with SMA is the etiology of diseases. While in MD the genetic origin directly affects the muscles, as a mutation in the dystrophin gene, in SMA the altered gene is in the motor neurons leading to muscle and anterior horn cell degeneration in the spinal cord. In both cases, a multidisciplinary team is required for treatment [2].

As previously mentioned, SMA is a disease that affects the locomotor system. Characterized as a rare neurodegenerative disease of autosomal recessive inheritance, SMA compromises skeletal muscle activity as a result of degeneration of medullary motor neurons of the nervous system. In patients with manifestation of the disease, the motor function linked to the activity of skeletal muscles is progressively compromised. The disease manifests differently and is classified into four types (SMA type I, type II, type III, and type IV) depending on the degree of muscle involvement and age at diagnosis [3]. Knowledge of skeletal muscles is fundamental for a better understanding of SMA and other muscle disorders.

2. Skeletal Muscle Tissue

Muscle tissue is formed by elongated cells of mesodermal origin and can be divided into three basic types with different characteristics: smooth muscle, cardiac striated muscle, and skeletal striated muscle [4]. The last type is the most abundant in the human body, has the capacity to convert chemical into mechanical energy, and enables rapid and vigorous voluntary contraction that ensures the consequent body movement [5].

The skeletal muscle tissue is composed of cylindrical, elongated, multinucleated cells surrounded by connective tissue (epimysium, perimysium and endomysium) and permeated by blood capillaries, lymphatic vessels and nerve endings. Each cell (myocyte) consists of filamentous bundles (myofibrils) formed by the repetitive arrangement of sarcomeres that contain actin and myosin filaments. In general, there are three main muscle fiber types present in this tissue. This classification can be type I, type IIA, or IIB. Type I fibers, also known as red fibers because of the high content of myoglobin and mitochondria, are characterized by a slower contraction and resistance to fatigue; type IIA fibers, are also characterized as red fibers, which use mitochondrial oxidation ATP, but have a rapid contraction and intermediate resistance; finally, type IIB fibers, also called white fibers, are characterized by rapid and potent muscle contraction, by the use of glycolytic metabolism in obtaining energy and by the low resistance to fatigue. Nevertheless, in some way and to a certain degree, these types of fibers overlap themselves, which contributes to certain plasticity [4], [6].

Muscle activation occurs from the neural plate where the neuromuscular junction and synaptic vesicles that store neurotransmitters are located. When the motor neuron receives a nerve impulse, acetylcholine – a neurotransmitter that depolarizes the cell membrane (sarcolemma) and triggers the secretion of Ca^{2+} ions stored in the sarcoplasmic reticulum – is released. The Ca^{2+} ions induce the interaction between actin and myosin filaments, which generates muscle contraction. The energy necessary for this contraction is derived from ATP and phosphocreatine, as well as from

muscle glycogen stores. Movement coordination is controlled by muscle spindles and Golgi tendon organs present in connective tissue and tendons close to the muscle, which capture changes in muscle fiber length and transmit the information to the central nervous system [4], [5], [7].

Returning to the model of SMA described earlier, this condition is caused by a mutation on chromosome 5q13, which can result in alterations or in the deletion of the survival motor neuron 1 (SMN1) gene, with a consequent reduction in the amount of SMN proteins. These proteins are responsible for maintaining the activity of motor neurons in the spinal cord. Deficient production of SMN proteins results in the degeneration of alpha motor neurons of the spinal cord, compromising the transmission of nerve impulses. The changes in the transmission of these impulses in the spinal cord culminate in an adaptive response of the muscle cell characterized by the progressive loss of its structural components and non-homogenous muscle atrophy. In its early stage, this disorder mainly causes symmetrical atrophy of the proximal muscles of the lower and upper limbs, with the legs being more affected than the arms; eventually, the trunk muscles are affected. Orthopedic and physiotherapeutic treatments designed to slow the progress of the disease are currently available; however, there is still no cure for SMA and no known means capable of recovering the already injured tissues exist [3].

In view of the scenario of approximately 13.3 million Brazilians with different types of motor disabilities, research aimed at the regeneration and recovery of skeletal muscle tissue has provided promising methods for the rehabilitation and improvement of the quality of life of this population [3]. The aim of this study is to perform a narrative review of the tissue engineering procedures applied to skeletal muscle regeneration using SMA as a model. The review was conducted following the principles described by Giorno et al. [8]. Searches were performed in the following databases: United States National Library of Medicine (MEDLINE) via PubMed; Latin American and Caribbean Health Sciences Literature (Lilacs); Cochrane Library; SciVerse Scopus; Web of Science, and Scientific Electronic Library Online (SciELO) through the Virtual Health Library (BVS). The following Health Sciences Descriptors were used: muscle regeneration; bioresorbable polymers; skeletal muscle; tissue engineering; spinal muscular atrophy.

2.1. Muscle injuries and regeneration

Skeletal muscle has limited ability to regenerate after injury, although the skeletal muscle fibers do not divide. The repair capacity of this muscle tissue is believed to be due to satellite cells [4], [9]. The function of this cell population has yet to be elucidated. It is known that satellite cells are located around skeletal fibers and that they are mitotically inactive in adults; however, they can resume the capacity of self-renewal and proliferation in response to certain stress or trauma [9].

In the case of more severe skeletal muscle injuries in which the capacity of tissue regeneration is lost [7], research in the area of tissue engineering is useful because it aims at finding methods to treat these injuries. Such studies focus on tissue repair and have a high potential for use in cell therapies [10].

The complexity of muscle tissue and the variety of cell types hinder the production of biomaterials capable of promoting full regeneration of this tissue. However, recent studies report that 3D cell culture models have shown promising results

in mimicking muscle structure, which would permit to create an extracellular matrix that stimulates the repair of injuries in the body [10]. These models permit the *in vitro* modeling of tissues. When inserted into the live organism, these tissues provide growth factors that promote the differentiation and organization of cells at the site of injury, permitting movement and strength recovery in patients with severe muscle injuries [11].

A series of methods for culturing isolated muscle cells on biomaterials allow the *in vitro* generation of a functional skeletal muscle with a morphology similar to the native muscle from porous substrates. One such strategy is the culture of myoblasts on three-dimensional fibrous or porous scaffolds composed of different materials, including fibrin and poly(e-caprolactone), often enriched with growth factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) [12].

In cases of SMA, treatments that exclusively target muscle functions, such as physical therapy and regular exercise, promote improvement in the impaired motor capacity of patients with this disease. Likewise, biopolymers with properties similar to skeletal muscle tissue developed in the laboratory also have the potential to stimulate motor rehabilitation in the tissue, not only preventing progression of the disease but also promoting recovery of the activity of the previously atrophied muscle [3].

3. Tissue Engineering

Tissue engineering is an interdisciplinary field aimed at the development of human tissues in the laboratory. Using biopolymers and extracellular matrices combined with *in vitro* cell cultures, this branch of biomedical engineering recreates functional organic tissues capable of regenerating injured tissues or mimicking their activities, minimizing possible side effects when inserted into the body [11], [13].

In addition to causing loss of function, muscle atrophies affect the esthetics and self-esteem of patients and reduce their quality of life. To minimize such impacts, surgeons seek to implant decellularized biological scaffolds through loco-regional or distant reconstructions; however, the patient's organism may trigger a process of fibrosis instead of myogenesis, as desired [14]. Therefore, the study and improvement of techniques used in tissue engineering that are capable of promoting muscle repair and regeneration are fundamental.

In skeletal muscle tissue, more complex injuries that exceed the body's regenerative capacity through the differentiation of satellite cells can be repaired using artificial muscle tissues created in *vitro*. Once transferred to the injured patient, the tissues developed in the laboratory interact with the organism without suffering marked immune rejection, although an inflammatory process must be present to a greater or lesser extent. These grafts can assume the function of cells with deficient activity in order to restore the activity of the locomotor apparatus [10].

Promising experimental results have been obtained with the use of scaffolds produced from elements of animal extracellular matrix. Decellularized extracellular matrices (dECM) have been shown to provide structural, biochemical and biocompatible support for the growth of new muscle cells in injured tissues. In view of the large three-dimensional

complexity of skeletal muscle tissue, these matrices can be produced from material obtained from tissues of biological donors [15], [16].

Experiments using porcine intestinal mucosa as dECM have reported good results, with the material promoting rapid cell differentiation and regeneration of muscle tissue in the abdominal wall of dogs, as well as adherence and incorporation of the matrix into the muscle. Better results were obtained when compared to synthetic polypropylene matrix also used in that study. The dECM promoted myogenesis of cells compatible with those of the original tissue to be regenerated and did not trigger immune rejection processes [15].

Other studies using dECM originating from muscle tissues have also reported promising results regarding the improvement of the regenerative capacities of skeletal muscle. For example, Perniconi et al. [16] conducted experiments with muscle tissue matrices *in vivo*, decellularized matrices *in vivo* and implanted into mice. The scaffold produced served as a biological matrix and was able to originate cells with a differentiation pattern of skeletal muscle tissue, in addition to promoting angiogenesis. In that study [16], dECM proved to be a pro-myogenic environment that was sufficiently strong to induce the myogenic specification of host stem cells, suggesting that the scaffold possesses the molecular properties of a niche for muscle differentiation.

Experiments using porcine urinary bladder tissues as dECM demonstrated the restoration of vascularization and myogenesis restricted to the area close to the injured site, but with no significant results regarding the remodeling of muscle shape and function [17]. This study [17] thus suggests that the use of decellularized matrices as a basis for tissue remodeling was only able to improve the internal function of the affected tissue through myogenesis when stem cells derived from the muscle were still present at the site of injury. According to the authors [17], the promotion of active regeneration of the shape and function of skeletal muscle tissue would occur through the production or migration of new muscle stem cells (satellite cells) to the dECM at the injury site.

The reported results were not very promising, probably because of the choice of matrix used since porcine urinary bladder tissues differ substantially from muscle tissues. In addition, after severe tissue injury, the fibrotic process triggered by the organism may provide a certain support to the muscle structure. Aurora et al. [17] also observed the formation of fibrotic tissue. However, this functional adaptation is sometimes related to the body's susceptibility to new injuries and occurs concomitantly with inflammation, which compromises muscle function [18].

In view of the structural complexity of muscles, cell migration, cell-cell interaction and cell proliferation are limited in injured tissues. Scaffolds composed of gelatin facilitate these processes. Using gelatin microfibers, MacQueen et al. [19] observed a behavior characterized by greater cell agglomeration in short fibers and more elongated cells in longer fibers. In addition to the importance of the characteristics of this scaffold, these results indicate that, besides tissue repair, gain in muscle volume is possible. These authors [19] used cells obtained from the smooth muscle of bovine aorta and from the skeletal muscle of rabbits that were cultured on gelatin fibers produced by rotary jet spinning. Both cell types showed good adhesion to the gelatin fibers, growing in a three-dimensional manner from the matrix and forming focal points of strong adhesion between the extracellular medium, membrane, and cytoskeleton. In the case of

skeletal muscle cells, the formation of elongated cells with an eccentrically located nucleus was observed [19]. Although the objective of the authors was the generation of muscle fibers for purposes other than tissue engineering, the results obtained may be applicable to regenerative medicine.

Hydrogels and gelatinous synthetic materials can be used as substitutes of extracellular matrices obtained from biological donors. These materials exhibit structural advantages when compared to dECM because they are developed according to the particularities of the tissue to be regenerated. As tested by Fuoco et al. [20], synthetic poly(lactic-co-glycolic acid) [PLGA] and poly(L-lactic acid) matrices exhibited good muscle regenerative capacity. Since it structurally resembles a hydrogel, the gelatinous scaffold ensures the support of the three-dimensional muscle tissue structure and facilitates the exchange of substances and stimuli that allow the regeneration of cells and muscle activities, in addition to exhibiting better adherence and incorporation of the regenerated structure into skeletal muscle [20]. These findings reinforce the possible therapeutic superiority of implantation of a scaffold versus direct cell injection into the injured area.

Boldrin et al. [21] injected myogenic cells into injuries of immunocompetent mice to avoid rejection, either alone or combined with a bioresorbable PLGA scaffold. The results showed signs of muscle regeneration in the cell implants with PLGA, with histological analysis revealing the presence of myogenic cells of human muscle fibers at the site of injection, inside and close to the implanted structure. On the other hand, the formation of fibrous tissue was observed where only cells were implanted [21].

In cases of patients with SMA, studies [3] have shown that therapies focusing on improving their motor capacity promoted greater vascularization of motor neurons, thus reducing mortality. Likewise, strengthening of the muscles is able to prevent and mitigate other complications caused by atrophy in general, such as scoliosis and gastrointestinal disorders, improving the quality of life of these patients [3]. Within this context, tissue engineering can be very promising in the treatment of this condition, as well as other motor disabilities. In theory, the regenerative capacity of the lost muscle might be an important therapeutic approach in conditions like SMA.

4. Conclusion

Methods developed by tissue engineering aimed at the repair of muscle injuries are important to improve the quality of life and to increase the autonomy of individuals with motor impairment. Within this context, techniques using bioresorbable materials, such as decellularized biological scaffolds and hydrogels, have shown promising results in the regeneration of skeletal muscles, contributing to the recovery of the motor capacity of individuals with physical disabilities.

5. Acknowledgement

The authors would like to thank the Dean of Research (ProPes) of Federal University of ABC (UFABC, Brazil) for the fellowship granted to ALLM.

REFERENCES

1. Censo Demográfico 2010: Características gerais da população, religião e pessoas com deficiência. In: IBGE. Rio de Janeiro, 2010. Accessed October 2020. [Online]. Available: https://biblioteca.ibge.gov.br/index.php/biblioteca-catalogo?view=detalhes&id=794
2. Iftikhar M, Frey J, Shohan MJ, et al. Current and emerging therapies for Duchenne muscular dystrophy and spinal muscular atrophy. Pharmacol Ther. 2021;220:107719.
3. Baioni MTC and Ambiel CR. Spinal muscular atrophy: diagnosis, treatment and future prospects. J Pediatr. 2010; 86:261-270.
4. Junqueira LCU, Carneiro J. Histologia básica. 12. ed., Rio de Janeiro, Brazil: Guanabara Koogan, 2013.
5. Trovato FM, Imbesi R, Conway N, et al. Morphological and functional aspects of human skeletal muscle. J Funct Morphol Kinesiol. 2016;1(3):289-302.
6. Exeter D and Connell DA. Skeletal muscle: Functional anatomy and pathophysiology. Semin. Musculoskelet Radiol. 2010;14(2):97-105.
7. Frontera WR and Ochala J. Skeletal muscle: A brief review of structure and function. Calcif Tissue Int. 2015; 96(3);183-195.
8. Giorno LP, Rodrigues LR, and Santos Jr AR. Métodos avançados para tratamento de queimaduras: uma revisão. Rev Bras Queimaduras. 2018;17(1):60-65.
9. Li EW, McKee-Muir OC, and Gilbert PM. Cellular biomechanics in skeletal muscle regeneration. Curr Top Dev Biol. 2018;126:125-176.
10. Sarrafian TL, Bodine SC, Murphy B, et al. Extracellular matrix scaffolds for treatment of large volume muscle injuries: A review. Vet Surg. 2018;47(4):524-535.
11. Urciuolo A, Serena E, Ghua R, et al. Engineering a 3D in *vitro* model of human skeletal muscle at the single fiber scale. PLoS One. 2020;15(5):e0232081.
12. Kwee BJ and Mooney DJ. Biomaterials for skeletal muscle tissue engineering. Curr Opi Biotechnol. 2017;47:16-22.
13. Santos Jr AR and Zavaglia CAC. Tissue Engineering Concepts. In Reference Module in Materials Science and Materials Engineering: Hashmi S (editor-in-chief), Oxford: Elsevier, 2016.
14. Wang HD, Lough DM, Kurlander DE, et al. Muscle-derived stem cell-enriched scaffolds are capable of enhanced healing of a murine volumetric muscle loss defect. Plast Reconstr Surg. 2019;143(2);329e-339e.
15. Clarke KM, Lantz GC, Salisbury SK, et al. Intestine submucosa and polypropylene mesh for abdominal wall repair in dogs. J Surg Res. 1996;60:107-114.
16. Perniconi B, Costa A, Aulino P, et al. The pro-myogenic environment provided by whole organ scale acellular scaffolds from skeletal muscle. Biomaterials. 2011;32:7870-7882.
17. Aurora A, Roe JL, Corona BT, et al. An acellular biologic scaffold does not regenerate appreciable de novo muscle tissue in rat models of volumetric muscle loss injury. Biomaterials. 2015;67;393-407.
18. Mahdy MAA. Skeletal muscle fibrosis: An overview. Cell Tissue Res. 2019;375(3):575-588.
19. MacQueen LA, Alver CG, Chantre CO, et al. Muscle tissue engineering in fibrous gelatin: Implications for meat analogs. N P J Sci Food. 2019;3:20.

20. Fuoco C, Petrilli LL, Cannata S, et al. Matrix scaffolding for stem cell guidance toward skeletal muscle tissue engineering. J Orthop Surg Res. 2016;11(1);86.
21. Boldrin L, Malerba A, Vitiello L, et al. Efficient delivery of human single fiber-derived muscle precursor cells via biocompatible scaffold. Cell Transplant. 2008;17(5);577-584.

Citation: Martins ALL, Santos AR Jr, and Giorno LP. Tissue engineering applied to skeletal muscle injuries: An overview of therapeutic perspectives. J Bio Med Open Access. 2021;2(1):121.