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Histopathological Study of Experimentally Crushed Skeletal Muscle's Regeneration in Adult Albino Rats

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Abstract

Aim: This study aims to explain all the events of skeletal muscle repair and regeneration with the help of suitable histophathlogical photomicrographs taken from crush-injured adult albino rat's gluteus maximus muscle. **Materials and Methods**: The present study is part of our previous research study related to skeletal muscle repair and regeneration in crush injured gluteus maximus muscle of adult albino rats. The samples were processed for histopathological examination using routine and special histological staining procedures. The tissue samples were examined under trinocular microscope, and the fields showing interesting findings were recorded under different magnification. **Results**: In this study we observed all regenerative changes in myofibers and related structures after crushed injury. **Conclusion**: Histopathological studies with good stainings are helpful for the easy identification of minute changes that occurs in each stages of skeletal muscle regeneration.

Keywords: Aldehyde fuchsin, Fast green, Myofibers, Picrosirius red, Satellite cells, Staining

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Introduction

In human and animals, the skeletal muscle is most abundant and widely distributed tissue and has innate capacity to repair and regenerate after injuries. Skeletal muscle is made up of multinucleated myofibers. The adult muscle stem cells are the satellite cells which are located in between sarcolemma and basal lamina.[1] Regenerative capacity of the satellite cells are essential in muscle repair after injury. The skeletal muscle regeneration occurs spontaneously in case of minor injuries whereas severe injuries alter the muscle healing which leads to fibrotic tissue formation.[2]

Frequently facing challenges with respect to skeletal muscle injuries are encountered in sports, accidents and during many surgical procedures. In sports injuries, the muscle damage occurs at myofibers level.[3] In mouse, rat and human, the overall dynamics of the muscle healing phases are quite similar.[4]

Many special histological staining methods are available to visualize and identify the connective tissue fibers. Some authors[5,6] reported that for the study of collagen fibers, the most powerful method is the picrosirius red staining procedure. In this study, we try to reveal good stainings which help to explain the fiber components of the extracellular matrix (ECM) and changes that occur in each stages of regeneration.

Materials and Methods

This study was part of another research study conducted in the Department of Anatomy, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Number of samples, method of surgical procedure, and sample preparations are described in our previous studies.[7-9]

For histopathological examinations, the routine (Hematoxylin and Eosin) and special histological staining procedures such as Masson's Trichrome (MT), PicroSirius Red with Fast Green (PSRFG), and Aldehyde Fuchsin with Fast Green (AFFG) were used. Photomicrographs with relevant findings were taken at different magnification under Trinocular microscope (Olympus, BX40; Japan) by a digital camera (Sony 18.2 MP, Japan).[7-9]

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Results

The observed microscopic changes in different phases of muscle repair process noticed in the present study were as under:

Degeneration

It was represented by hypertrophied and hypereosinophilic myofibers, degenerated myofibers with multinucleated giant cells, undulated sarcolemma, necrotic fibers with mineralization, swollen, vacuolated, hyalinized and fragmented myofibers and hemorrhages [Figure 1].

Inflammation

The observed inflammatory changes were in the form of infiltration of inflammatory cells [Figure 1] and fatty depositions were seen in connective tissue coverings [Figure 1].

Regeneration

In the longitudinal sections of myofibers, the regeneration was characterized by the presence of activated satellite cells that

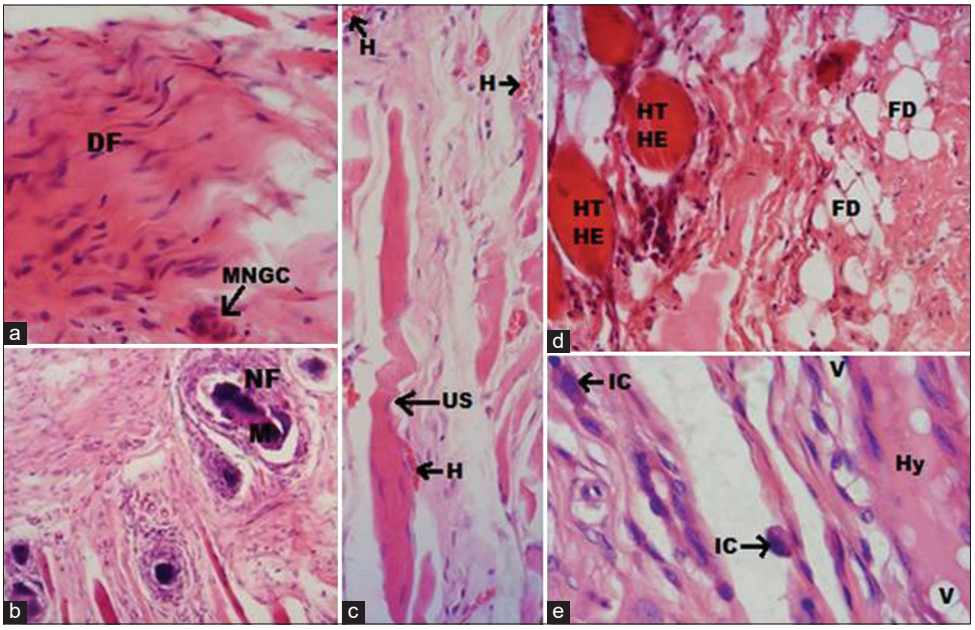

Figure 1: Representative images of longitudinal sections. (a) DF: Degenerative fibers, MNGC: MultiNucleated Giant Cell. (b) NF: Necrotic fibers, M: Mineralization. (c) US: Undulated sarcolemma, H: Hemorrhages. (d) HTHE: Hypertrophied and hyperesonophilic fibers, FD: Fatty Depositions. (e)V:Vacoules, Hy: Hyalinization, IC: Inflammatory cells. Stain: Hematoxylin and Eosin. Initial magnifications of all images except image (b) are at ×400 and b is at ×200

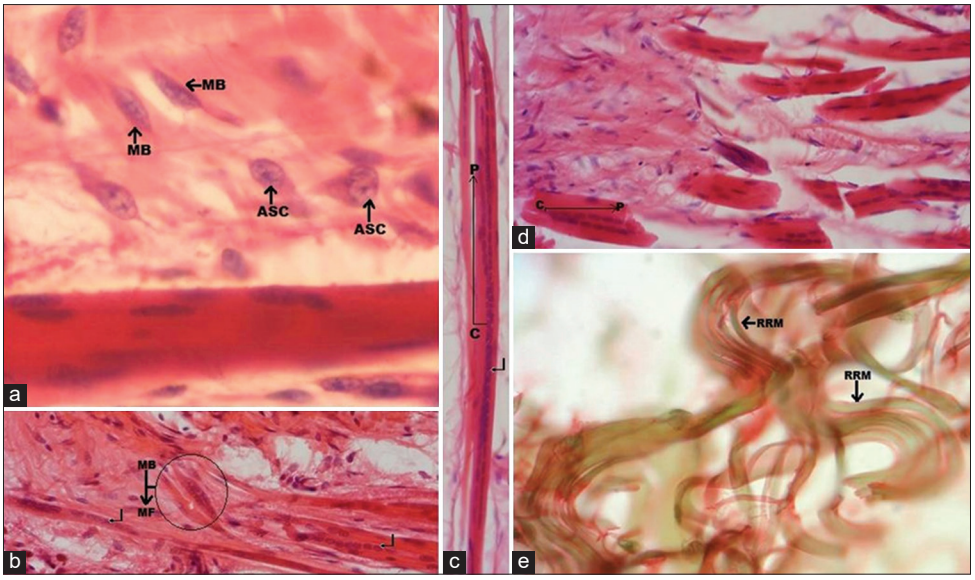

Figure 2: Representative images of longitudinal sections. ASC: Activated satellite cells, MB: Myoblast, MB→MF: Myoblasts fused to form myofiber, ◴: Row of vesiculated myoblasts' nuclei in the center of regenerating fiber, C→P: movement of myonuclei from center toward periphery, RRM: Recently, regenerated myofibers. Images- Hematoxylin and Eosin stained section (a) and PicroSirius Red with Fast green stained section (e) at ×1000 of magnifications and (b,c and d) Hematoxylin and Eosin stained sections at ×400 of initial magnifications

differentiated into myoblasts with row of vesiculated nuclei in the center of regenerating fibers and the movement of centrally placed myonuclei to the periphery in recently regenerated myofibers [Figure 2]. In the transverse sections of myofibers with centrally placed nuclei, movements of these nuclei from center toward periphery [Figure 3] and split fibers [Figures 3 and 4] were also noticed.

Remodeling

The main features of remodeling observed were as follows:

Connective tissue remodeling

All connective tissue coverings contain both collagen [Figure 5] and elastin fibers [Figure 6].

Neovascularization

Numerous newly formed blood capillaries were found in all connective tissue coverings and also in relation with the regenerated myofibers [Figures 4 and 5].

Maturation/Functional Repair

Functional repair and maturation of newly regenerated myofibers were marked by the presence of regenerated nerve bundles specially in their epimysial connective tissue coverings [Figures 4 and 5].

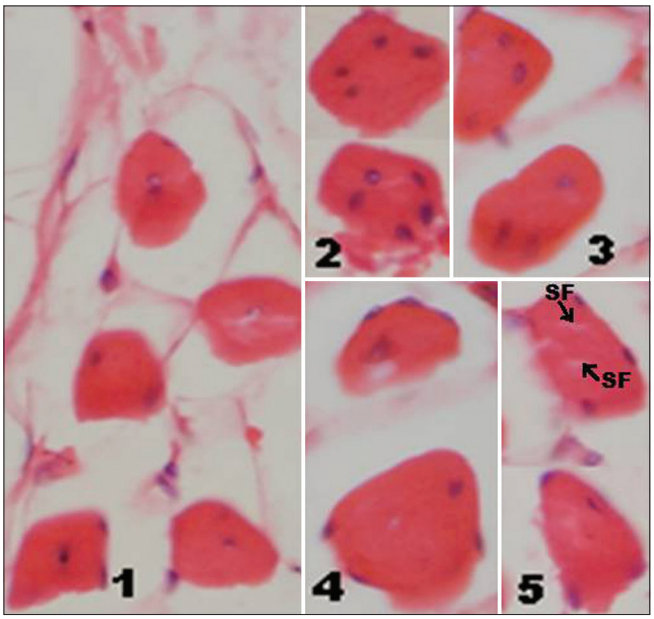

Figure 3: Representative images of transverse sections (1, 2, 3, 4 and 5) showing the different position of myonuclei from center to periphery, SF: Split fibers. Stain: Hematoxylin and Eosin at initial magnification ×400

Discussion

Skeletal muscle regeneration events occur in five sequential, overlapping and time-dependent phases such as degeneration, inflammation, regeneration, remodeling, and maturation/functional repair.[4,10] Muscle regeneration process starts in the 1st week by the activation and differentiation of satellite cells and maturation of the myofibers.[11] The activated satellite cells is an integral part of repair and regeneration process.[12] The activated satellite cells can proliferate and differentiate into myoblasts which replace the damaged fibers and also give additional myonuclei to their parent myofiber.[13]The initial stage is the muscle degeneration which occurs within the first few hours after injury. The changes in this stages are cell necrosis, disruption of the myofibers, sarcolemma, hematoma formation, and debris clearance.[14-16] In necrosis, the features in the enlarged myofibers are altered internal architecture, altered plasamalemma permeability, uncontrolled influx of calcium ions, and an increased number of mononucleated cells.[4,10,13] The degeneration features were histologically identified as necrotic fibers with mineralization, disrupted and undulated sarcolemma, hypertrophied and hypereosinophilic myofibers, hemorrhages, swollen, vacuolated, hyalinized and fragmented myofibers [Figure 1].

At certain levels, the coordinated activity of inflammatory cells, resident myogenic and non-myogenic stem cells and connective tissue fibroblasts also help in muscle repair after damage.[11,16-18] However, chronic inflammatory response always interfere the regeneration process by inhibiting the physiological activity of stem cells.[10] In this histopathological study, the routine staining method reveal the presence of inflammatory cells mainly the macrophages [Figure 1].

The adipokines secreted by the adipose tissues are said to attract the macrophages to the injured area,[19] these cells help in the removal of debris and activate the satellite cells.[20,21] Absence or alteration of the macrophages' response leads to the adipose tissue deposition within the muscle.[22] In this present study, adipose tissue deposition was noticed within all connective tissue coverings [Figure 1].

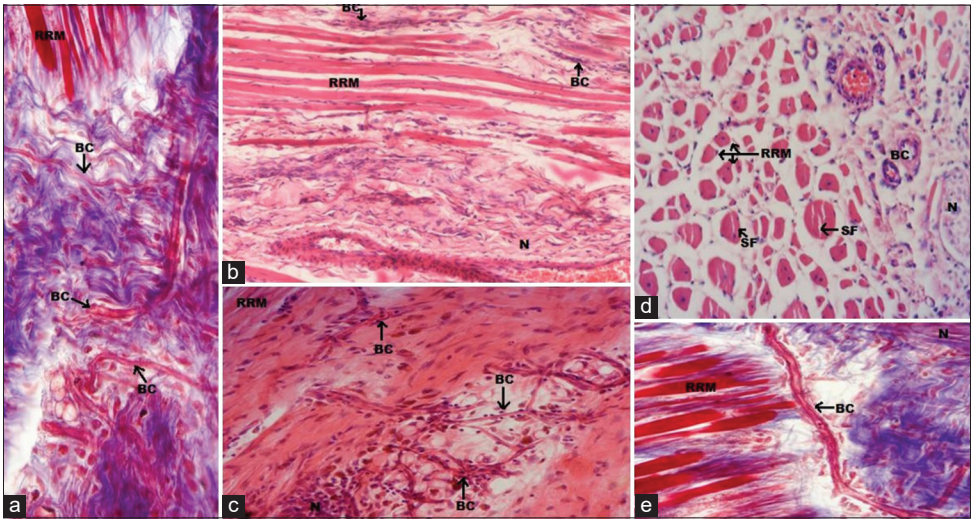

Figure 4: Representative images of longitudinal sections (a,b, c and e). Representative image of transverse section (d). RRM: Recently Regenerated Myofibers, BC: Blood Capillaries, N: Nerve bundle, SF: Split fibers. Stains: images a and e- Masson's Trichrome, images (b, c and d) Hematoxylin and Eosin. Initial magnifications- images A, c and e at ×400, B at ×100 and D at ×200

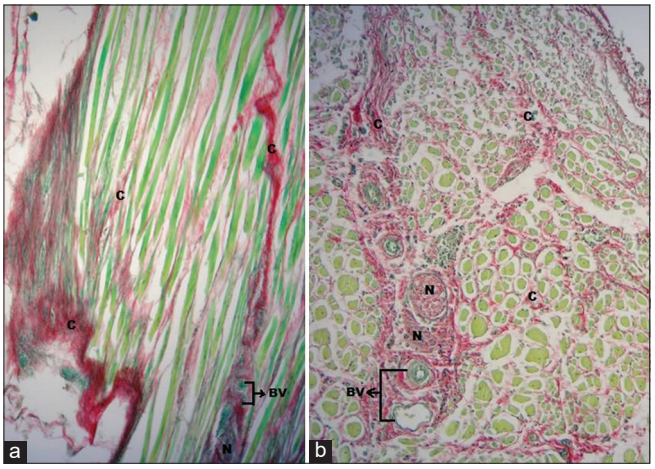

Figure 5: a: Representative image of longitudinal section, b: Representative image of transverse section. BV: Blood Vessels, N: Nerve bundle C: Collagen fibers (red color). Stain: PicroSirius Red with Fast green. Images: (a) initial magnification at ×100 and (b) at ×200 of magnification

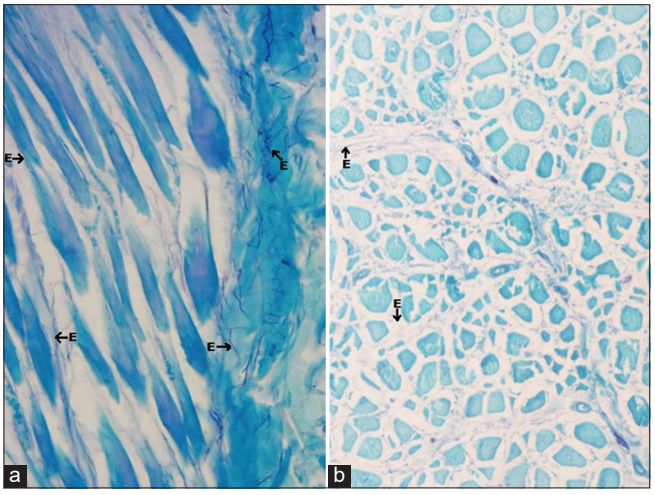

Figure 6: a: Representative image of longitudinal sections, b: Representative image of transverse section. E: Elastin Fibers (violet color). Stain: Aldehyde Fuchsin with Fast green. Images: (a) initial magnification at ×400 and (b) at ×200 of magnification

Some newly regenerating myofibers revealed split, which is considered to be due to the incomplete fusion of regenerating fibers within the same basal lamina [Figures 3 and 4].[23,24] The presence of centrally nucleated regenerating myofibers indicated the fusion-mediated muscle regeneration process.[25] The peripheralization of nuclei in myofibers indicated the formation and maturation of new myofibers[4,26] which can be seen in many photomicrographs [Figures 2 and 3]. Thus, in this histopathological study, all characteristic features of myofibers regeneration process and different stages of satellite cells were observed.

Connective tissues in the ECM is a fundamental requirements for myoblast migration and fusion.[27] The stromal fibroblasts and myoblasts produce the ECM and collagen fibers. This ECM contains elastin, laminin, fibronectin and proteoglycans.[22,28] Appropriate deposition of components of ECM is essential for successful muscle regeneration and loss of its any components can lead to myopathy.[18] In general, the fibrotic response is beneficial during the initial stages of muscle regeneration because it stabilizes the tissues, gives support and strength and provide protection to the injured site. However, the excessive and persistent fibrin deposition within the injured area often leads to heavy scarring and creates hindrance in the normal muscular functions.[2,10,18] In this present study, collagen fibers' [Figure 5] depositions were well identified by Picrosirius red with fast green staining. The elastin fibers [Figure 6] were noticed using Aldehyde fuschin with fast green staining.

In addition, during muscle repair process, both the angiogenesis and myogenesis occur simultaneously. At the injury site, the blood vessels are ruptured that leads to the tissue hypoxia.[15] Therefore, increased muscle capillarity is essential to improve blood-tissue exchange properties and that helps to maintain the tissue remodeling.[29] Newly formed blood capillaries at the injured site is essential for the growth and maturation of the regenerating myofibers.[11,18] In routine and special histological stainings, numerous newly formed blood capillaries were observed in relation to regenerating myofibers [Figures 4 and 5].

The regenerated myofibers attain the maturity and functional recovery only after successful reinnervation. Nerve supply can also directly influence protein turnover and gene expression within multinucleated regenerating myotubes and indirectly influence the proliferation and differentiation of satellite cells.[30-32] Reinnervation first begins in the surrounding area of injured site and then it grows toward the newly formed myofibers.[11] In this study, special histological staining methods also proved helpful for identification of reinnervation in relation to regenerating myofibers [Figures 4 and 5].

Conclusion

Muscle injuries commonly occur during various accidents, sports and as a result of surgical procedures. The special staining procedures are useful for the identification of changes that occurs in regeneration process. Especially, collagen fibers deposition in the extra cellular matrix can be easily distinguished by PicroSirius Red with Fast Green staining and elastin fibers by Aldehyde Fuchsin with Fast Green staining.

Conflict of Interest Statement

We declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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References

1. Standring S. The Anatomical Basis of Clinical Practice. 41st ed. Amsterdam, Netherlands: Elsevier, Churchill Livingstone; 2016. p. 103.
2. Laumonier T, Menetrey J. Muscle injuries and strategies for improving their repair. J Exp Orthop 2016;3:15.
3. Counsel P, Breidahl W. Muscle injuries of the lower leg. Semin Musculoskelet Radiol 2010;14:162-75.
4. Forcina L, Cosentino M, Musarò A. Mechanisms regulating muscle regeneration: Insights into the interrelated and time-dependent phases of tissue healing. Cells 2020;9:297-1324.
5. Lattouf R, Younes R, Lutomski D, Naaman N, Godeau G, Senni K, *et al*. Picrosirius red staining: A useful tool to appraise collagen networks in normal and pathological tissues. J Histochem Cytochem 2014;62:751-8.
6. Séguier S, Godeau G, Brousse N. Collagen fibers and inflammatory cells in healthy and diseased human gingival tissues: A comparative and quantitative study by immunohistochemistry and automated image analysis. J Periodontol 2000;71:1079-85.
7. Elsy B, Khan AA, Maheshwari V. Effects of d-α-tocopherol on skeletal muscle regeneration in crushed injury of diabetic rats. Eur J Anat 2017;21:293-304.
8. Elsy B, Khan AA, Maheshwari V. Regenerative potential of d-δ-tocotrienol rich fraction on crushed skeletal muscle of diabetic rats. J Interdiscip Histopathol 2017;5:36-42.
9. Elsy B, Khan AA, Maheshwari V. Co-administered Vitamin E isoforms d-α-tocopherol and d-δ-tocotrienol rich fraction promote regeneration of skeletal muscle in diabetics. Int J Nutr Food Sci 2018;7:47-55.
10. Musaro A. The basis of muscle regeneration. Adv Biol 2014;2014:612471.
11. Ceafalan LC, Popescu BO, Hinescu ME. Cellular players in skeletal muscle regeneration. Biomed Res Int 2014;2014:957014.
12. Badr SM, Elbakary RH, Laag Em, Sarhan NI, Elbakary NA. Histological Study of the Effect of Platelet Rich Plasma on Experimentally Induced Skeletal Muscles Injury in Adult Male Albino Rats. The Egyptian J Histo. 2019, 369-79.
13. Karalaki M, Fili S, Philippou A, Koutsilieris M. Muscle regeneration: Cellular and molecular events. *In Vivo* 2009;23:779-96.
14. Tidball JG. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005;288:R345-53.
15. Jarvinen TA, Jarvinen TL, Kaariainen M, Kalimo H, Jarvinen M. Muscle injuries: Biology and treatment. Am J Sports Med 2005;33:745-64.
16. Yang W, Hu P. Skeletal muscle regeneration is modulated by inflammation. J Orthop Transl 2018;13:25-32.
17. Milner DJ, Cameron JA. Muscle repair and regeneration: Stem cells, scaffolds, and the contributions of skeletal muscle to amphibian limb regeneration. Curr Top Microbiol Immunol 2013;367:133-59.
18. Mann CJ, Perdiguero E, Kharraz Y, Aguilar S, Pessina P, Serrano AL, *et al*. Aberrant repair and fibrosis development in skeletal muscle. Skelet Muscle 2011;1:21-40.
19. Bouloumie A, Curat CA, Sengenes C, Lolmede K, Miranville A, Busse R. Role of macrophage tissue infiltration in metabolic diseases. Curr Opin Clin Nutr Metab Care 2005;8:347-54.
20. Brunelli S, Rovere-Querini P. The immune system and the repair of skeletal muscle. Pharmacol Res 2008;58:117-21.
21. Rigamonti E, Zordan P, Sciorati C, Rovere-Querini P, Brunelli S. Macrophage plasticity in skeletal muscle repair. Biomed Res Int 2014;2014:560629.
22. Sciorati C, Clementi E, Manfredi AA, Rovere-Querini P. Fat deposition and accumulation in the damaged and inflamed skeletal muscle: Cellular and molecular players. Cell Mol Life Sci 2015;72:2135-56.
23. Blaveri K, Heslop L, Yu DS, Rosenblatt JD, Gross JG, Partridge TA, *et al*. Patterns of repair of dystrophic mouse muscle: Studies on isolated fibers. Dev Dyn 1999;216:244-56.
24. Bourke DL, Ontell M. Branched myofibers in long-term whole muscle transplants: A quantitative study. Anat Rec 1984;209:281-8.
25. Charge SB, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. Physiol Rev 2004;84:209-38.
26. Yin H, Price F, Rudnick MA. Satellite cells and the muscle stem cell niche. Physiol Rev 2013;93:23-67.
27. Lewis MP, Tippett HL, Sinanan AC, Morgan MJ, Hunt NP. Gelatinase-B (matrix metalloproteinase-9; MMP-9) secretion is involved in the migratory phase of human and murine muscle cell cultures. J Muscle Res Cell Motil 2000;21:223-33.
28. Gillies AR, Lieber RL. Structure and function of the skeletal muscle extracellular matrix. Muscle Nerve 2011;44:318-31.
29. Bloor CM. Angiogenesis during exercise and training. Angiogenesis 2005;8:263-71.
30. Mozdziak PE, Pulvermacher PM, Schultz E. Muscle regeneration during hind limb unloading results in a reduction in muscle size after reloading. J Appl Physiol 2001;91:183-90.
31. Mitchell PO, Pavlath GK. Skeletal muscle atrophy leads to loss and dysfunction of muscle precursor cells. Am J Physiol Cell Physiol 2004;287:1753-62.
32. Slater CR, Schiaffino S. Skeletal muscle repair and regeneration. In: Advances in Muscle Research. Berlin, Germany: Springer; 2008. p. 303-34.