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Evaluation of Histology Change of Skeletal Muscle Regeneration in Adult Rabbit

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Abstract

Skeletal muscles are the biggest cells in the human body, the chief purpose of skeletal muscles are drive motion. Renewal is procedure that depend on skeletal basement membrane, satellite cell (are precursors to skeletal muscle cells and are responsible for the ability of Sample of 50 mature male rabbits ere used in this study, the muscle tissue to regenerate.). body weight range between (2 .5 -3) kg . Animals were separated randomly into three sets (set 1 control group contain of 10 animal , set 2 contain of 20 animal at 3rd post-operative week , set3 contain of 20 animal at 6th post-operative week). Evaluation the morphological changes that happen in regeneration and degeneration were selected the extensor digitorum longus muscle and examination after 21days and 42 days post -operative . renewal of skeletal muscle characterizes an vital homeostatic process of skeletal muscle in adult.In development, the capability of skeletal muscle to regenerate is response to different injured stimuli, restoring damaged myofibers. Repair of muscle fibers is dynamic process include regeneration and degeneration , in the case of degeneration the muscle fibers not It is not complete recovery but in the regeneration the myoblast . The skeletal muscle consist of myofibers formed by myoblasts(mononucleated)the myoblast has the principle role in growth of muscle fibers and development

Introduction:

Skeletal muscle consist of elongated cylinder fibers , each fibers has multi nuclei located in the peripheral ,muscle fibers of skeletal muscle has striated apperance The reason is back into myocine and actine arrangement. Skeletal muscle fibers has good ability in the regeneration because have stem cell called myosatellite cells(myogenic precursor cells) in the normal stage these cell is quiescent state when muscle exposure to in injury the cells transformed into active state (Birbrair et al., 2013).

after injury the myosatellite cells undergo numerous stage of proliferation then differentie into myoblaste , the myoblast fuse together or with infected muscle fibers to accomplished the muscle regeneration (Yablonka-Reuveni, 2011) .Light injury lead to minimum differentiate of satellite cells but trauma cause maximum proliferation in precursor stem cells ,fuse myoblast is crucial in regeneration process also this fuse contribute in the growth the muscle fibers. (Ciciliot, & Schiaffino, 2010),(Lehto et al., 1985) skeletal muscle repair is significant process and overlapping between infiltation of inflammatory cells and satellite cells activation and

transfer into myoblast(Musaro, 2014).The regeneration of skeletal muscle succeful if muscle fibers recover ,restoration in all type of blood vessles,extra cellular matrix and repair muscle fibers orient with original orientation of fibers. (Huijbregts et al.,2001).

Materials and methods:

Animals for experimental :

1-In this study take forty male rabbit wilt body from 2-2.5 kg .Each animal taken go under general anesthesia by using ketamine and xylazine (35mg/kg and 5mg/kg) respectively relies on the direction National Institutes of Health guidelines. The doses calculated by the formula bellow

The calculated dose = (weight x exact dose) divided by the concentration

2-The surgical operation accomplished under supervision of surgical department in Baghdad University , the site of injury sterilized by povidone iodine

3-The extensor digitorum longus was recognized after removing deep fascia and subcutaneous fascia and surrounded muscles

4-The extensor digitorum longus muscle was incise transversely (figure 1 and 2) also direct return to its location. The tendon of this muscle was cut from one side, and then after the tendon of origin of the extensor digitorum longus muscle was completely removel (figure 3).

5- Extensor digitorum muscle suture with absorbed suture , then suture the skin and limit movement of animal by using half split for seven days and give animals antibiotic for three days

6-post operative 21 -42 days take the extensor digtorum longus muscle under general anesthesia

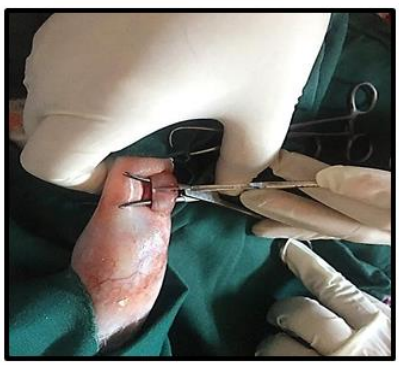


Figure (1): Shows the procedure of cutting the Extensor digitorum longus muscle.

Figure (2): Shows the site of operation (pointed by the scalpel) in Extensor digitorum longus muscle cut one tendon.

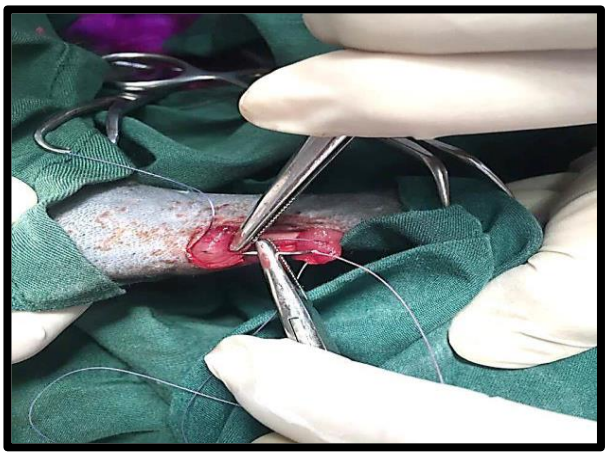


Figure (3): Shows during cut the Extensor digitorum longus

Collection of experimental samples

This study design 3 sets of animals

Set 1	Set2	Set3
10 extensour digtorum longus muscle take from animals in control stages	20 extesour digtorum longus muscles taken from removed of animals at end of 21 days post-operative	20extesour digtorum longus muscles removed of animals at end of 42 days post- operative

Protocol to examine the extensor digtorum longus muscle using hematoxylin and eosin H&E)(

The extensor digitorum longus muscle were prepared for paraffin section as follows :

1- Fixation the EDLM fix in Formalin-Fixed Paraffin Embedded 10% for twenty four hours

1- Dehydration: is carried out by immersing EDLM in a runs of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol is reached(70%-100%)

2- Clearing: clearing agent is xylene, and several variations are necessary to totally displace ethanol. would be:

xylene 20 min

xylene 20 min to ensure a good transparency.

2-Wax infiltration and embedding : is the last stage ,the EDLM can be infiltrated by a suitable paraffin wax, this process occur by 2 steps :

1- The first step: mix the xylene with wax for one hour.

2- The second step: the EDLM place in the absolute paraffin wax for two hours . The finishes the trip from tissue to wax block

3- Sectioning by microtome.: thin sections(seven micron) for electron microscopy , These thin sections were transmitted to a water bath (temperature 40- 45C^{o}) were placed on glass slides for Hematotoxyline and eosin stain.

4- De-waxing and Rehydration:

Achieved by treating the section with xylene for 30 minutes and pass the section during descending concentrations of ethanol baths (100%, 90% and 70%) for 5 minutes, then washed with distilled water.

5- Staining the extensor digtorum longus muscle by hematotoxyline and eosin:

*The staining process by H&E:

1-the EDLM after remove the wax and hydrated

2-apply the alum hematoxylin for three minutes

3-Wash the slide with (four –five) changes of tap water until blue color stops coming off slides

4-then stained the slide by 1% eosin yellowish for thirty second .

3- Wash by tap water for one minute.

4- Dehydrate by three changes of 95% ethyl alcohol and two changes of 100% ethyl alcohol for one minute each and mounted by permount (DPX).

Results:

General histological changes of skeletal muscle:

In this study the regeneration of skeletal muscle by using autography procedures in two different stage , the first stage in 21 days of post –operative and second stage in 42 days of post –operative. The extensor digitorum muscle is located in the posterior forearm of animal and human, figure (4) show the site of injury in the first stage appear narrow and irregular but figure (5) show the muscle regeneration after 42days of post- operative is complete the process of regeneration . Few cases of muscle regeneration end by formation scar tissue and fail in regenerations process

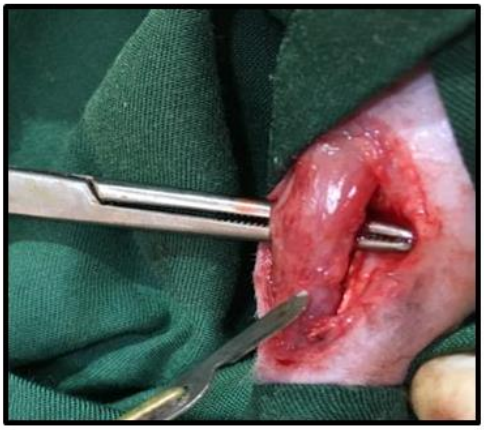


Figure (4): show the first stage of the skeletal muscle regeneration in 21 days after post-operative the site of injury narrow and irregular

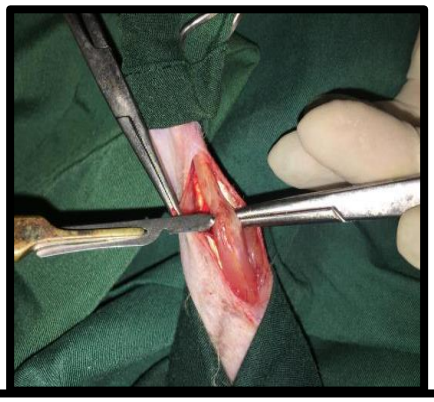


Figure (5): show the second stage of skeletal muscle regeneration in42 days after post-operative appear the site of injury is complete the process of regeneration.

Cellular dynamic changes of skeletal muscle regeneration :

skeletal muscle possesses a unique ability to regenerate, this process occur in 3 stages :

1- Revascular of blood vessels .
2- Re- establish (epimysium, perimysium ,and endomysium)
3- Regeneration of muscle fibers

in the early process of regeneration may be degeneration process observed , this process occur by the inflammatory cells rapidly invade into injured muscle tissue and release cytokines to activation the other inflammatory cells , these cells take different morphological pattern (Figure 6 A,B). at the beginning of the first stage of regeneration including the formation of myofibrillar and appear mononuclear inflammatory cell infiltrate and show fascicles surrounded by connective tissue (figure 7) , in first stage (21days) of regeneration noticed degeneration process and regeneration at site of injury , in degeneration process the different forms inflammatory cells present in the site of healing this cell located between connective tissue surround muscle fibers also show small blood vessels (Figure 7). Progressive weakness of degeneration of skeletal muscles it ended in end of 21 days , the tissue histological section devoid of different type of inflammatory (Figure 8). Restoration of the vascular supply is the first sign of regeneration. The regeneration process include neoformation of vessels surround the site of injury and formation numerous number and different type of blood vessels in wound site and area near to the wound (Figure 8 & figure 9). When the degeneration process diminishes this means that the inflammatory cells started to disappear at the same time Rebuilding torn muscle fibers (Figure 10& 11) . The early stage of the regeneration process is characterized by the appearance of new small muscle fibers mono-nucleate will differentiate to form multinucleated muscle fibers (Figure 12 & 13). The myoblast is a progenitor cell differentiate into a myocyte (muscle cell) of the skeletal muscles It is diffuse spread in connective tissue , (Figure 14).In the early stage of myogensis some of myoblast divide and developed into cylindrical muscle fibers characterized by multinucleated these fibers called myotube (Figure 15). Due to high degree of vascularization and suitable cellular environment in site of injury the space between the myotube full by connective tissue (Figure16& Figure 17). by the end of 42 days We can hardly notice the mature muscle fibers characterized by striated and multinucleated these nuclei located at periphery , (Figure18). The new muscle fibers have small size and nuclei located in the center with progress of regeneration process the muscle fibers are enlargement and the nuclei displacement into peripheral (Figure 15,16). at the end of 42 days of regeneration process represent by appear the endomysium separate muscle fibers one from other , perimysium is layer of connective tissue separate the muscle fibers into fascicles , and epimysium is outer layers of connective tissue surround the entire muscle fibers (figure17). not all the regeneration process end Success some of cases appear degeneration with mononuclear inflammatory cell in the site of injury also area near of the injury also some of cases end by seen scar tissue (Figure 18) . In failure of regeneration the ended by appearance fibrosis in the site of injury , the fibrosis prevents the muscle regeneration and the chief reason in muscle weakness (19).in the other hand, in degeneration it was observed small foci of new small fibers and proliferation of fibroblast (Figure 20&21).

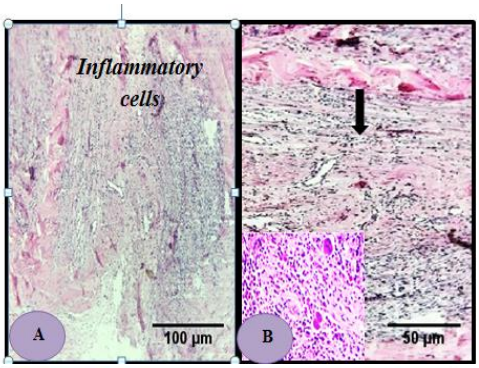


Figure (6) cross section in regenerating skeletal muscle showing **A:** heavy mononuclear cells infiltration at the site of the injury with few intact peripheral muscle cell fibers, X (10) **B:** higher magnification of same picture heavy cells (H&E 1st weeks, X 10, 20,100).

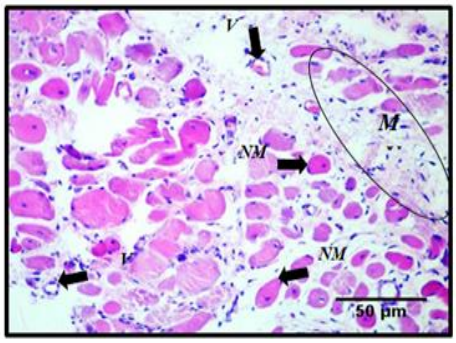


Figure (7) cross section in regenerating muscle after injury showing the formation of new muscle fibers (NM), high vascularity (V) &still few mononuclear cells (M) are present in the scene

(H&E, 21 days X 20).

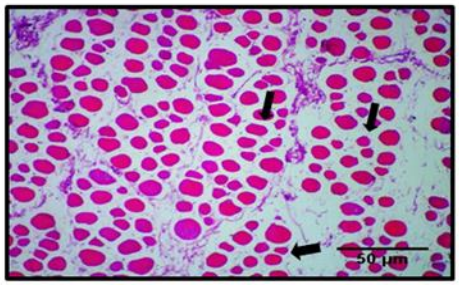


Figure (8): cross section in regenerating muscle showing newly formed small muscle fibers and disorganized connective tissue elements
(H&E, 21 days , X 20).

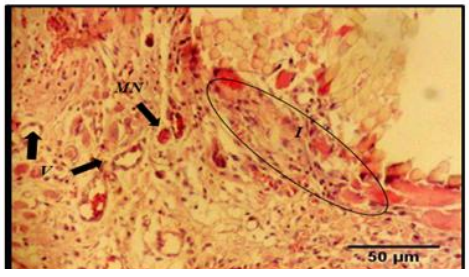


Figure (9): cross section in regenerating muscle showing the presence heavy vascularity (V) together with appearance new muscle fibers (NM) notice the persistence of mononuclear inflammatory cells (I) (H&E, 21 days , X 20)

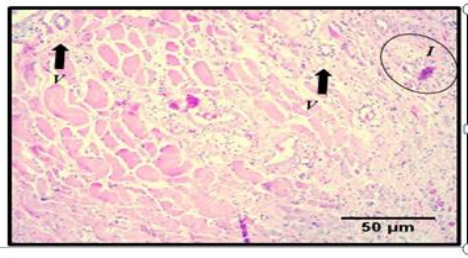


Figure (10): cross section in regenerating muscle showing the site of injury will heavy vascularization (V) and new muscle fibers appearance at the site with few inflammatory cells (I)
(H&E 21 days, X 20.

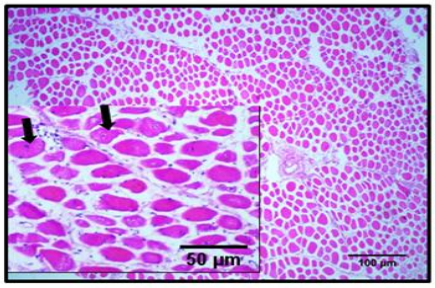


Figure (11): cross section in regenerating muscle showing well organized newly form muscle fibersblstt still they have centrally located nuclei (H& E , 21 days, X10,20)

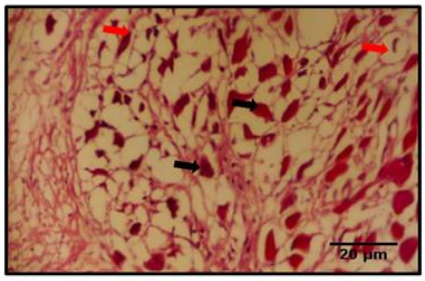


Figure (12): cross section in regenerating skeletal muscle showing appearance of myoblast(➡) and very early new muscle fibers (NM)(➧) at the site of injury after clearance of area from debris (H&E 21 days, X 40).

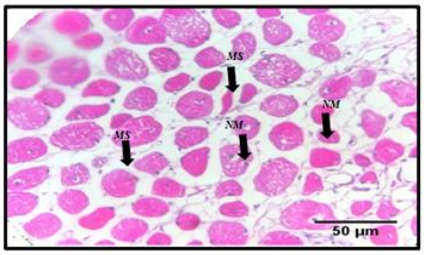


Figure (13): cross section in regeneration muscle fibers showing the appearance myoblast (MS) and newly formed muscle fibers(NM) (H&E 21days , X 20).

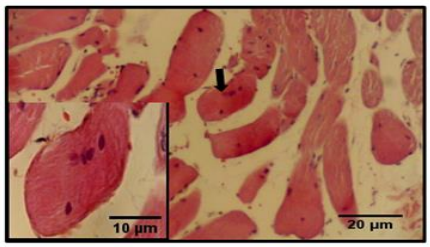


Figure (14): cross section in regenerating muscle showing different forms of myotubes with nuclei starting to arrange in row (H&E, 21 days X 40.100).

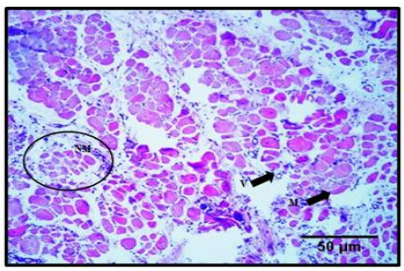


Figure (15): cross section regenerating muscle showing dynamic process degeneration & regeneration where we can see vascularity(V) , new muscle fibers(NM) , early myotube (M) and inflammatory cells(I) (H&E, 21 days , X20).

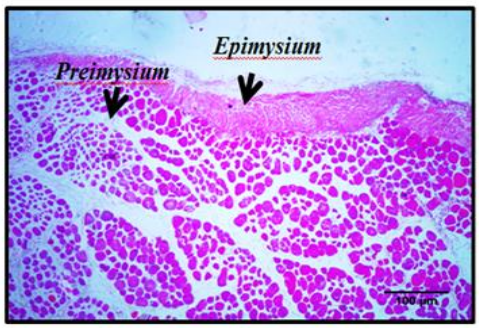


Figure (16): cross section in regeneration muscle fibers showing the distribution of connective tissue with well identified epiysium ,but perimysium & are still not well organized
(H&E 21 days X 10).

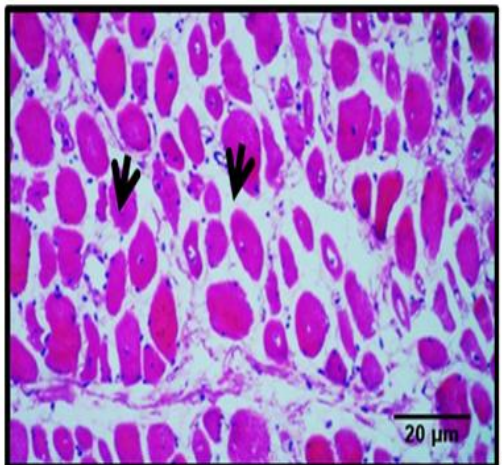


Figure (17): cross section in regeneration skeletal muscle higher magnification of previous images showing no organized perimysium (P)& clear type 2C fibers can be seen (H&E, 21 days , X 40).

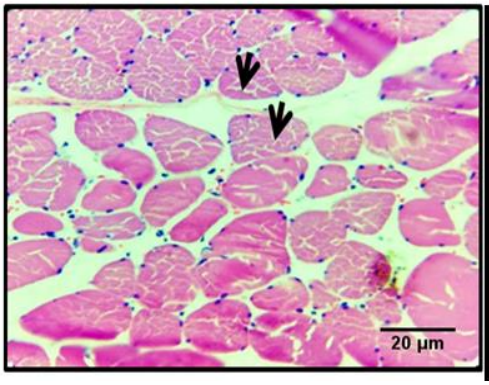


Figure (18): cross section in regeneration skeletal muscle showing the settlement of myofilament (in sarcoplasm where the cells reached maturity at42 days after injury
(H& E., 42 days, X 40).

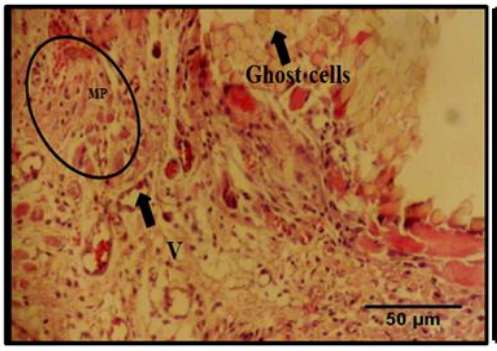
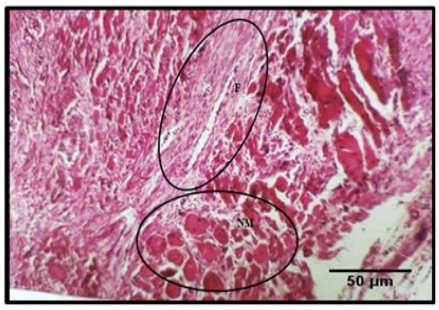


Figure (19): cross section in regeneration skeletal muscle showing failure of regenerative process with high MP cell, vessels & the appearance of Ghost cell

(H&E. 42 days , X 20 .



Figure(20): cross section in regeneration skeletal muscle shoeing formation scar fibrosis of tissue, (F) still small foci of regenerating muscle fibers are present (NM) (H&E, 42^{days}, X 20) .

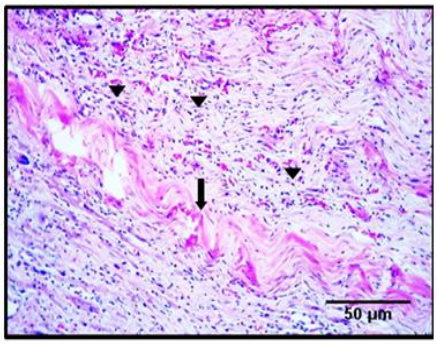


Figure (21): cross section in regeneration skeletal muscle showing scar formation at the site injury after failure of regeneration. Notice the presence of heavy collagen bundles (⬇) with high vascularity (▼)
(H&E, 42^{days}, X 20)

Discussion

In vivo experimental injury a models for regeneration:

Skeletal muscle is important structure in human body ,these cells have many function ,responsible of movement of bones in skeleton .its consist of myosin and actin give the muscle striate appearance (Holmberg& Durbeej , 2013).

the injury of musculoskeletal system is most common type in during sports ,for example , in football players ,usually occurs in muscle of the lower limbs theses injury effect on functional ability.Although skeletal muscle disorders painful seldom lead to fatal (Robertson et al.,1993).

The traumatic of skeletal muscle has ability to start biological response to stop more muscle loss ,the minor of injury occur in the muscular system heal automatically in adults . Some cases can use external physical inducement by sports medicine (Tedesco et al., 2010). The types of transplants are four 1-xenograft2- isograft 3- allograft4- autograft , but the best sample to project for muscle injury is done in living body these type of study called vivo

Events occurs in skeletal muscle fibers during healing and regeneration:

The time schedule of the main events during muscle regeneration are essential and form an overlapping schedule and superimposed process these stages included in regeneration of injured muscles are revascularization reinnervation and lastly establishment of tendon connection the mention above process are crucial stages for muscle regeneration (Schiaffino et al ., 2013).

in this study showed different stages of renewal skeletal muscle new t t found types of epithelial pale cells when stain the slides by hematotoxyline and eosin , these not have nucleus with eosnophilic cysto plasma those characteristic give the cell translucent appearance , these cells called ghost cells . The inflammatory cells that invade the site of injury in early

stage of regeneration, these cell effect on muscle fibers behaviors during regeneration (Ciciliot & Schiaffino 2010).

.Myonecrosis is lead to disorder organization of muscle fibers , with Z line loss, mitochondrial swelling and sarcolemmal disruption. The factor that maintenance of integrity skeletal muscle fibers is sarcolemma .necrosis and degeneration found inside the line of injury , these lesion must be diagnosis and assessment dangerous degree (Musaro, 2014).

the new fibers muscle formed after 21 days of postoperative , these cell characterized by small nucleus and located in center , the muscle fibers consist of thick filament and called type 2c fibers, It has been noticed increase in size and the nucleus displace into peripheral .when stain by hematotoxyline and eosin for tracking type 2c fibers it's have features regeneration fibers, and characterized by basophilic cytoplasm, vesicular nuclei (Nonaka,1991)and (Li&Huard,2002)

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