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Research Article

The Effect of A Contusion Injury on Rabbit Skeletal Muscle: A Morphological Study

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Abstract.
The paper describes the effects of two massage modalities on uninjured skeletal muscle morphology by the light microscopic evaluation of healthy vervet monkey and rabbit muscle. It remains unknown how treatments may influence the morphology of injured muscle during the process of healing. To determine this, the "drop mass" technique was employed to create a measurable injury to the left *vastus lateralis* muscle of five New Zealand white rabbits. Biopsies were obtained 6 days after injury from the juxta-bone and sub-dermal regions of the healing left and uninjured (control) right *vastus lateralis* of each animal. The tissue was fixed in formal saline, embedded in wax, cut, stained with haematoxylin and eosin and phosphotungstic acid haematoxylin and examined by light microscope. While the morphology of the muscle from both juxta-bone and sub-dermal biopsies remained abnormal 6 days after injury, a simple scoring system devised to describe the "severity" of residual morphological abnormality revealed juxta-bone tissue to be the most traumatised. The "drop-mass" technique appears to cause a contusion within a single muscle of at least two degrees of severity.

Keywords: skeletal muscle; contusion injury; light microscopy; morphology

1. Introduction

Few studies have reported group has reported the effects that two physiotherapeutic modalities, deep transverse friction [1, 2] and compressed air massage (CAM) have on rabbit *vastus lateralis* muscle and healthy vervet monkey *tibialis anterior* muscle [3, 4] To date, however, it has not been possible to determine how or whether either modality may influence the physiological mechanisms of healing following an injury. This report is part of an ongoing study primarily designed to create a reproducible trauma, whose effects could be measured at a fixed period after injury. The results of such a study could be used as a baseline against which various physiotherapeutic modalities could be compared.

From the literature, the simplest and most easily adjusted method of producing a contusion injury to skeletal muscle has been by dropping weights from various heights through a guide tube onto a limb. By adjusting weight and height of drop the investigator has control over the degree of injury. Furthermore, by placing the tube over a predetermined area/muscle, injury can be localized to a particular region. Examples of this methodology abound. Crisco et al [5] dropped a 171 g weight from a height of 102 cm onto the mid-belly of the posterior surface of the *gastrocnemius* muscle of mice. Minamoto et al [6] injured 17 rats by dropping a 200 g weight from a height of 37 cm on to the middle belly of the *tibialis anterior* muscle. Bunn et al [7], dropped 100 g or 200 g weights from a height of 130 mm through a guide tube located over the *quadriceps* muscle of mice. Physiotherapists have employed the drop-mass technique to study the effect of various therapeutic regimens. Fisher et al [8] dropped a solid aluminium bar with flat surface of 1.38 cm^2 weighing 700 g down a tubular guide through a distance of 125 mm on to the medial gastrocnemius muscle of rats. Their purpose was to determine the effects of laser therapy on acute blunt trauma.

Most studies that employ the drop-mass technique to effect injury have used small rodents. This study has focussed its attention on the effect that different forms of massage had on rabbit *vastus lateralis* or vervet monkey *tibialis anterior* muscle. In order to provide an adequately sized muscle mass and to comply with our earlier studies, the New Zealand white rabbit was selected as our animal model. In a prior study, we modified the drop-mass technique employed by Bunn et al [7] on mice to create a reproducible injury in rabbits that was evident six days after injury [9] (under review for publication). The same technique was employed in this study where a weight of 201 g was dropped seven times from a height of 100 cm on to the *vastus lateralis* of New Zealand white rabbits.

The aim of this study was to report the appearance of healing muscle six days after contusion injury. A simple scoring system was devised to describe the "severity" of residual morphological abnormality. It is hoped that the results can be later employed to create a quantifiable baseline against which various therapeutic modalities used to treat injured muscle may be compared.

2. Material and Methods

Five New Zealand, white rabbits (R1–R5) were studied with the approval of the Ethics and Research Committees of the University of KwaZulu-Natal. The animals were housed in a barrier animal facility in the animal research facilities of the University of KwaZulu-Natal and fed *ad libitum*, commercial rabbit pellets. They were maintained under the care of the staff of the Biomedical Resource Unit of the University of Kwazulu-Natal according to the "Approved Standard Protocols" set by the Animal Ethics Sub-committee of the University of KwaZulu-Natal.

The day before procedure, the hind limbs of the animals were shaved and hair further removed with commercial hair removal product (No Hair®Adcock Ingram Healthcare (Pty) Ltd.) to facilitate observation of any inflammatory reaction and facilitate treatment. Before injury, all animals were weighed and anaesthetised with an intramuscular injection of the combination of 30 mg/kg body weight (bw) ketamine (Anaket-V, Bayer (Pty) Ltd. Isando South Africa) and 4 mg/kg bw xylazine (Xylavet 2%, Intervet SA (Pty) Ltd. Isando South Africa) followed by subcutaneous injection of 5 mg/kg bw morphine sulphate (Morphine sulphate-Fresenius PF 10 mg/ml Bodene (Pty) Ltd. Port Elizabeth South Africa). In a side lying position, the shaven upper thigh of the rabbit's left hind limb was examined, and by palpation the mid belly of the *vastus lateralis* muscle identified. To ensure that the injury was applied to the same position, the site of injury was marked with indelible ink. To ensure a reproducible injury of consistent magnitude, a guide tube, 100 cm in length was placed directly over the mark and an elongated oval weight of 201 g dropped on the muscle seven times. The point of impact was calculated to be approximately 4 mm^2.

Six days after injury the skin was incised longitudinally over the lateral mid-thigh, the fascia opened and the muscle examined. Two degrees of injury were visually apparent: quite severe near the bone and less severe just beneath the skin. Wedge biopsies approximately 8 mm in length and 5 mm in diameter were taken from sub-dermal (SD) and juxta-bone (JB) positions within the *vastus lateralis* muscle from each injured left limb of animals R1–R5. To serve as morphological controls, biopsies from similar positions were taken from the uninjured right limbs of animals R2–R5. The animals' skin was sutured after biopsy and they were monitored for any post-operative discomfort during and after recovery from the anaesthetic. Prior to suture removal, the wounds were regularly cleaned with 2% hibitane solution.

All biopsies were bisected, a small portion of the specimen being placed in Karvovsky's fixative [10] for 1 hour prior to dehydration and embedding in epoxy resin. The larger sample was immediately immersed in 10% formal saline for 24 hours, dehydrated through increasing concentrations of ethanol, prior to clearing in xylene and embedding in paraffin wax. Using glass knives, sections, 1 μm in thickness were cut of the resin embedded material and stained with 1% alkaline Toluidine blue to provide resolution images of cellular entities. Sections, 4 μm in thickness were cut and stained with haematoxylin and eosin (H&E) to show general cellular features, and phosphotungstic acid haematoxylin (PTAH) to identify damaged or necrotic myofibres.

The sections were examined using a Nikon Compound Microscope (Nikon Eclipse 8i) and digital images of the tissue were captured at X10; X20 & X40 magnification and stored in jpeg format. The state of the muscle 6 days after injury indicated, by inference, the severity of the initial injury. To assist in this assessment, a simple numeric system was employed that assigned a score to morphological features associated with residual muscle injury and phase of repair (Table 2).

3. Results

The animals were mobile after recovery from the anaesthetic, immediately after the injury and within 12 hours of biopsy. Two weeks after surgery, the wounds had completely healed with no apparent ill effects being demonstrated by any

Table 1: The numerical scoring system of the morphological appearance of the muscle 6 days after injury.

Score	**Feature 1: Macroscopic appearance at time of biopsy**
0	No evidence of injury
1	Mild reddening of muscle tissue - mild haematoma
2	Macerated soft tissue with haematoma
	Feature 2: Epimysium
0	Normal - thin with occasional fibroblasts
1	Many fibroblasts in thickened epimysium
2	Many fibroblasts in very thickened epimysium with mono-nuclear cells invading muscle body
	Feature 3: Appearance of myofibres near epimysium
0	Normal myofibres in normal bundles
1	Some irregular myofibres in irregular bundles
2	Few if any myofibres-cellular region with? myoblasts, fibroblasts and myotubes
	Feature 4: Appearance of myofibres distant from epimysium
0	Normal myofibres in normal bundles
1	Abnormal myofibres in muscle bundles

animal. Some animals were noted to have bitten at the sutures but this did not interfere with healing and recovery.

There was a light reddening of the skin, ecchymosis, immediately following the seven hits in all animals which was absent by the time of biopsy, six days later. After incising the skin and prior to biopsy, it was noted that there was slight bruising of the muscle immediately beneath the impact zone in all animals and haemolysed muscle close to the bone, especially in rabbits R2–R5. Locating the *vastus lateralis* beneath the skin marker and by palpation was successful, as on visual inspection of the exposed muscles of the thigh, injury was confined to the target muscle. While the muscle from the control right limbs was normal in every respect (Figure 1), there was evidence of trauma both macroscopic and microscopic in the muscle from all injured animals.

Figure 2 illustrates the thickened epimysium (E) extending into the myofibres comprising the "muscle body" (B). The arrows indicate the rounded myofibres at the interface and the B. Microscopy revealed that in all animals, the muscle in the JB region had been quite severely damaged. In the immediate vicinity of the epimysium, there was a non-vascular region extending up to 3 mm into the muscle body.

In Figure 3, the region is characterised by the absence of mature myofibres and numerous mononuclear cells, some of which appear to be organising into myotubes (My) and/or capillaries (C). The numerous fibroblasts are indicated by arrows.

In Figure 4 there are occasional very small myofibres distributed through the cellular matrix. The arrows indicate small regenerating fibres in the cellular matrix.

Figure 5 shows the vacuolated and injured myofibres at the periphery of the cellular matrix. At the periphery, of this region, are swollen, sometimes vacuolated, generally rounded myofibres that are either exhibiting a continuing process of necrosis or undergoing repair.

Figure 6 shows thickened epimysium (E) within which are numerous fibrocytes and numerous mononuclear cells migrating into the interfibre spaces (arrowed). Beyond this region, the muscle bundles were normally perfused with capillaries and most myofibres appeared morphologically normal. The epimysium was markedly thickened in JB regions and contained numerous fibroblasts and other mono-nucleate cells, some of which appeared to be migrating into the muscle body.

The SD region, while showing evidence of trauma, was notably less traumatised than muscle in JB biopsies. Myofibres in the immediate vicinity of the epimysium were similar to those described at the periphery of the non-myofibrous, highly cellular matrix in JB samples above (Figure 5). In these samples, the muscle tissue was perfused with capillaries and the interfibre spaces contained numerous mononuclear cells. Beyond this region, which stretched up to 3 mm from the epimysium, the myofibres appeared morphologically normal. In rabbit 4, however, there was a thin (± 2 mm), severely damaged juxta-epimysial region similar to that seen in JB specimens (Figures 2–4). The epimysium appeared thickened and more cellular in all SD specimens, especially in rabbit 4 whose epimysium was similar to that shown in Figure 6.

The numeric scores awarded to the "severity" of morphological abnormality of biopsies from each SD and JB specimen are shown in Table ??. Note that the average score for SD samples was 3.4 and for JB samples 6.6. The mean scores are statistically significantly different. Mann-Whitney test $P = 0.0079$ with alpha set at 5%.

4. Discussions

Little work has been undertaken on how physiotherapeutic regimens provide relief from pain or help repair skeletal muscle after injury. The researchers have previously has

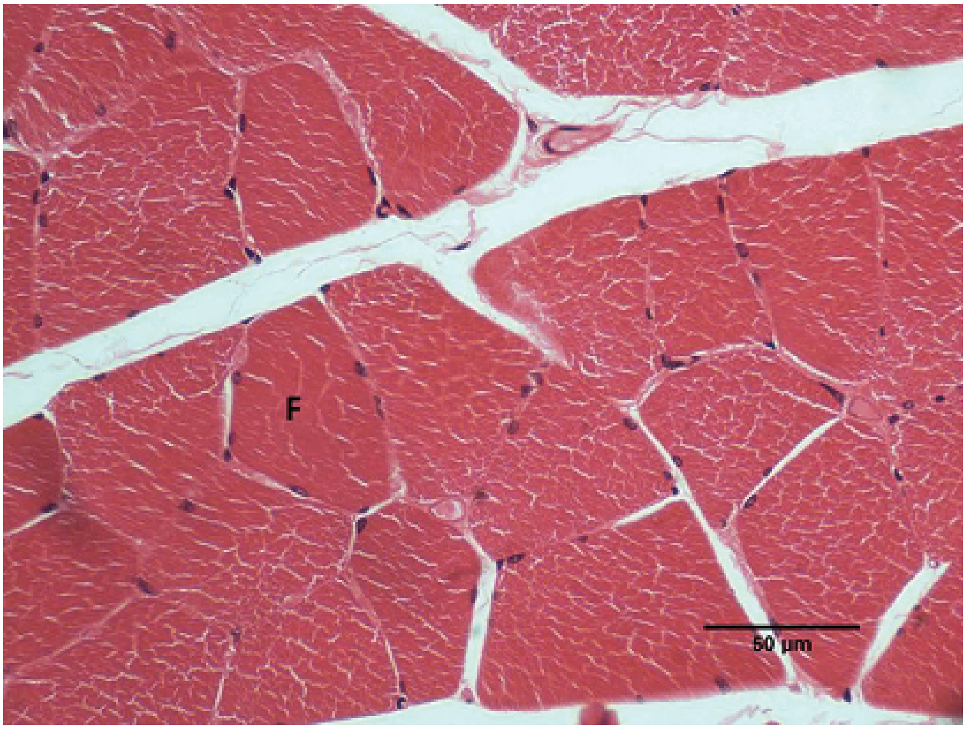

Figure 1: H&E stained section from JB biopsy: cross-sectioned myofibres (F) in untraumatised muscle.

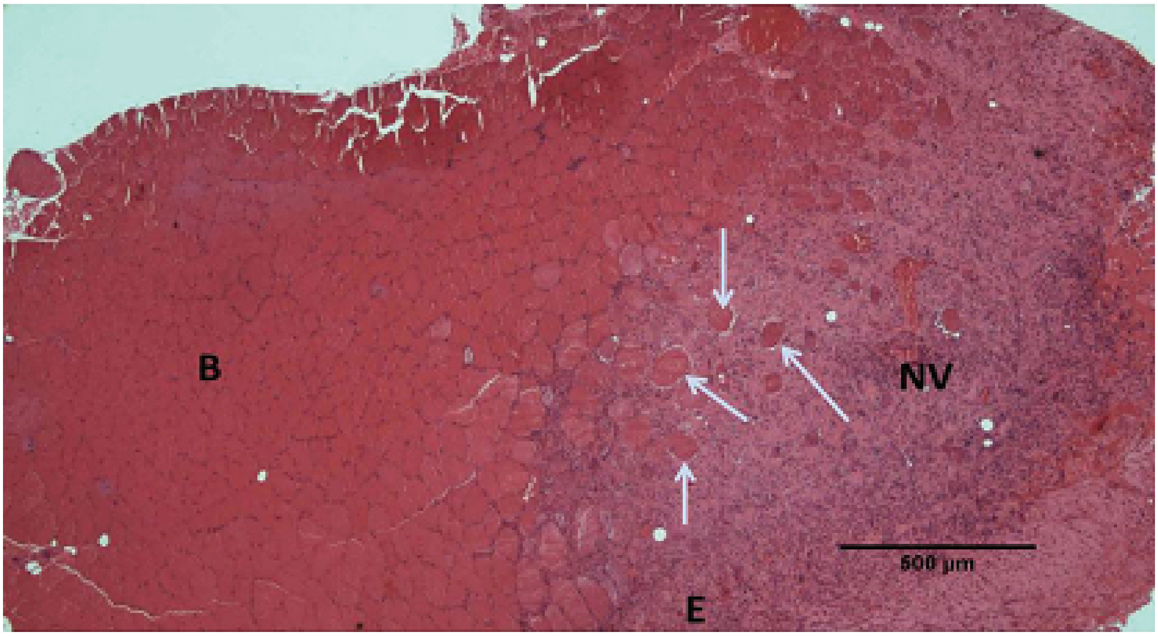

Figure 2: H&E stained section JB biopsy: a nonvascular, cellular area (NV).

investigated the mechanisms by which two therapeutic regimens may work—deep transverse friction [1, 2] and compressed air therapy [3]. The work was carried out on the healthy, non-injured muscle of vervet monkeys and rabbits. To determine how either of these or other physiotherapeutic modalities may influence healing after injury, it is necessary to create a reproducible, measurable trauma against which the morphology of tissue following a similar injury treated with a physiotherapeutic modality can be compared.

In this study, our primary objective was to produce and describe the morphological appearance of a reproducible injury in the rabbit *vastus lateralis* muscle. In an earlier study,

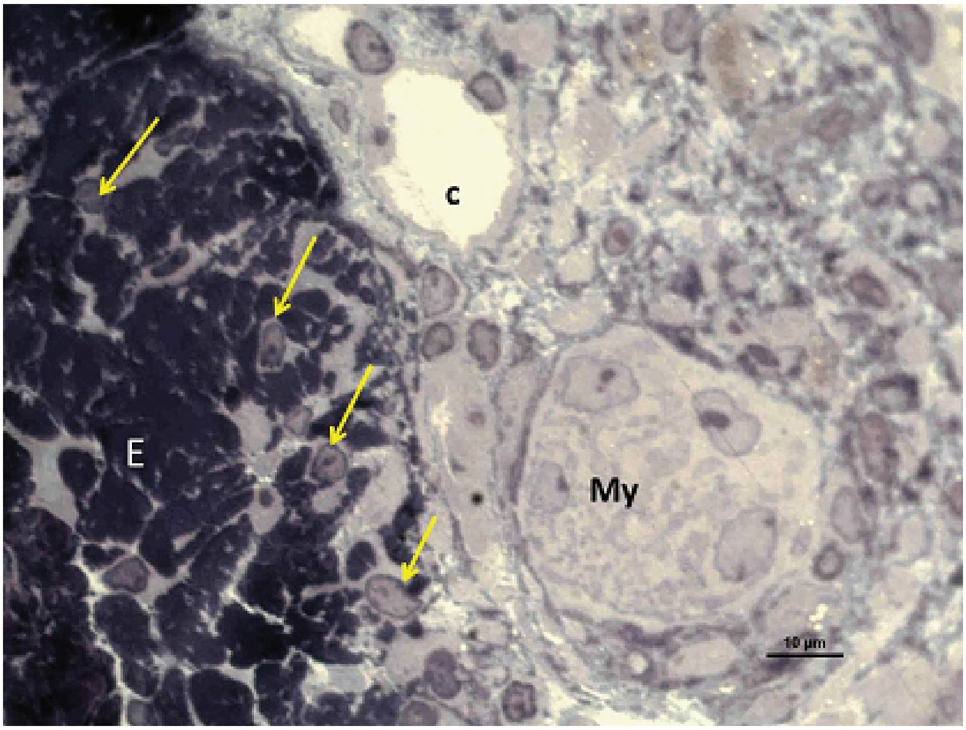

Figure 3: Toluidine blue stained section JB biopsy: mononuclear cells organising into myotubes (My) and capillaries (C).

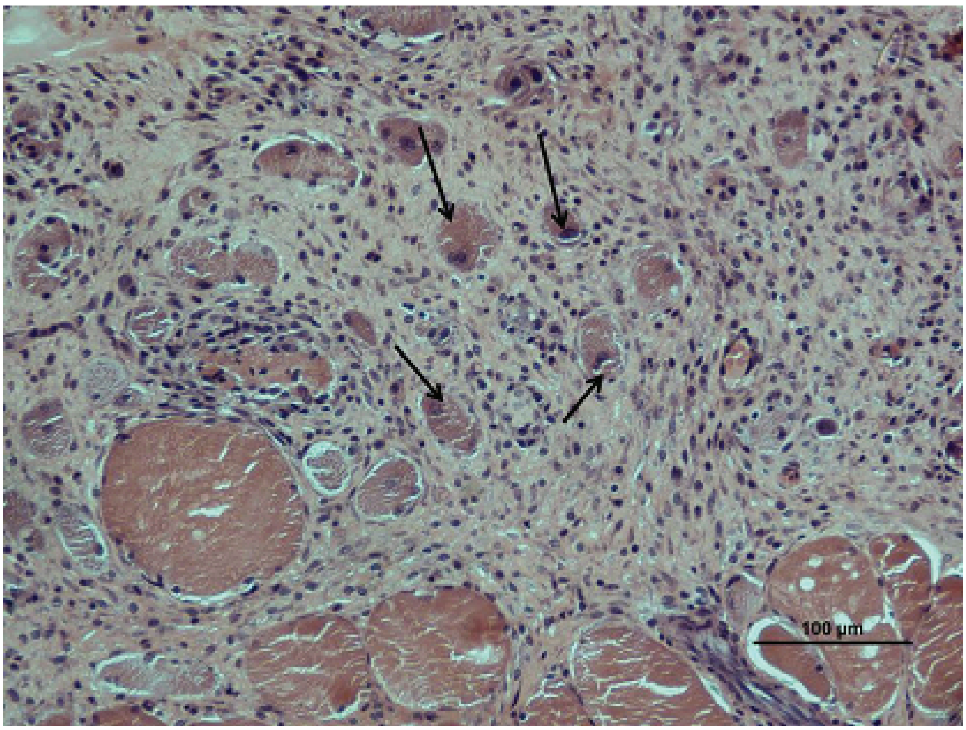

Figure 4: H&E stained section from JB biopsy: small regenerating fibres in cellular matrix.

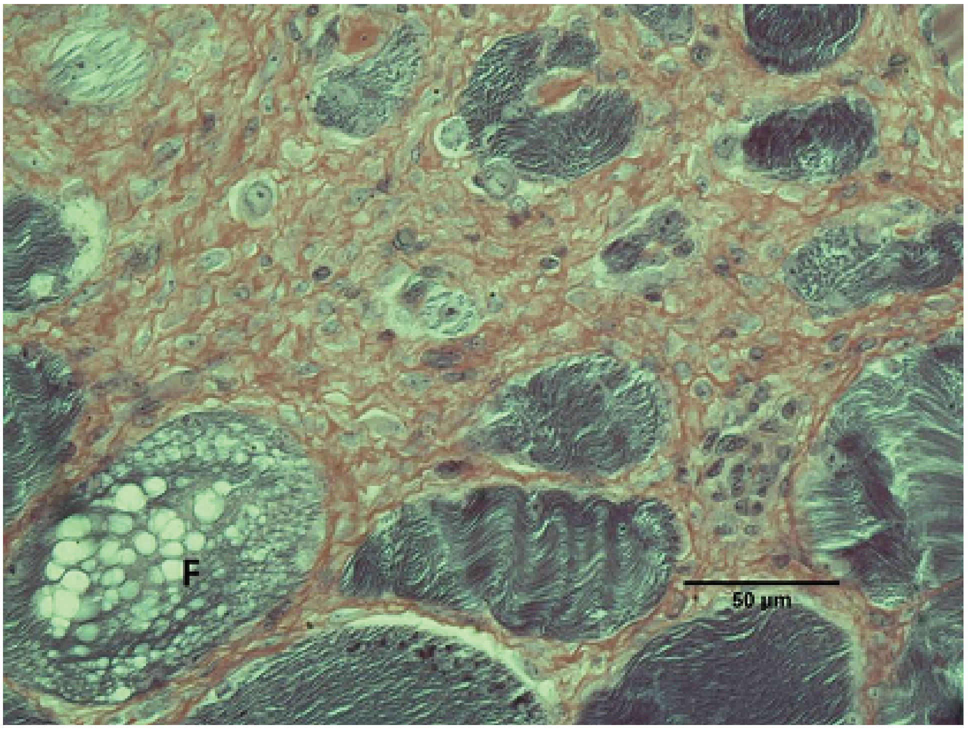

Figure 5: PTAH stained section from SD biopsy: vacuolated and injured myofibres at the periphery of the cellular matrix.

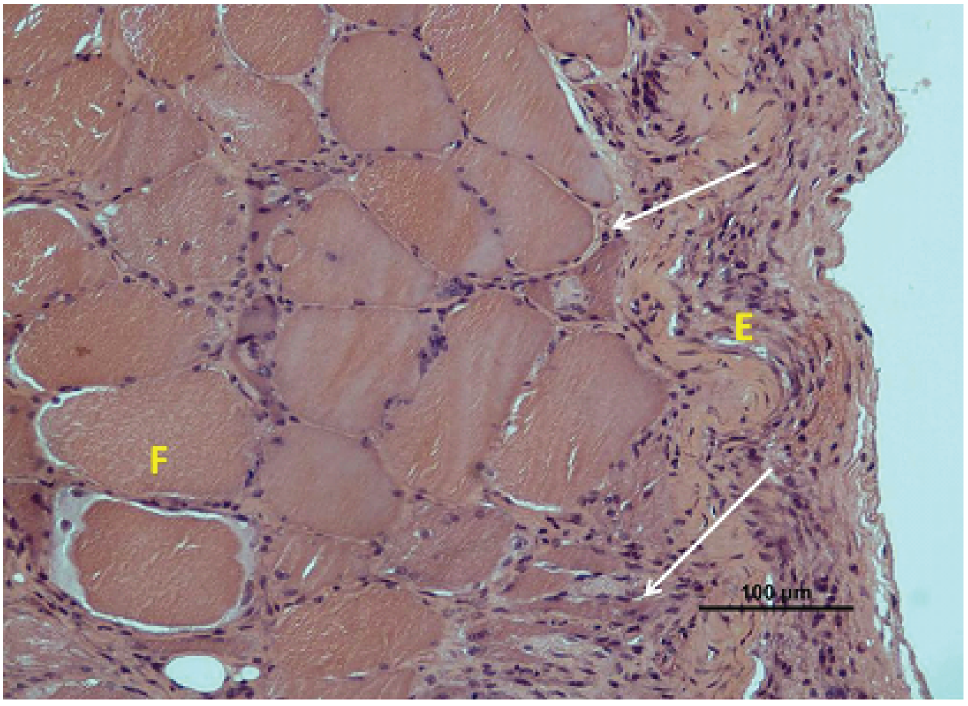

Figure 6: H&E stained section from SD biopsy: Thickened epimysium within which are numerous fibrocytes.

by trial and error, it was found that dropping a 201 g weight seven times from a height of 100 cm on to a pre-determined position of the *vastus lateralis* produced a contusion injury that showed residual signs of trauma six days after injury [9]. In the latter study, it was found that two levels of trauma were caused from a single contusion- apparently more severe near the bone (JB) and lesser injury in the sub-dermal (SD) position. The results of the study confirm both the

Table 2: Scores for F1-F4 of Superficial (SD) and deep (JD) biopsies.

Rabbit	F1	F2	F3	F4	Total SD	Total JB
1SD	1	1	1	0	3	
1JB	2	1	2	1		6
2SD	1	1	1	0	3	
2JB	2	2	2	1		7
3SD	1	1	1	0	3	
3JB	2	1	2	1		6
4SD	1	1	2	0	3	—
4JB	2	1	2	1		6
5SD	1	1	1	1	4	
5JB	2	3	2	1		8
				Mean	3.4	6.6

reproducibility of this method of injury and the variability of degree of injury in the muscle, for with the exception of the SD biopsy from rabbit 5, scores for each SD and JB biopsy are positionally similar. The anomaly in rabbit 5 could be ascribed to a number of variable—slight error of position of impact over the bone, thickness of muscle or simply the normal variation in individual physiological response to impact. It should be remembered that subjects do not all respond in exactly the same way to trauma, pharmaceutical intervention etc, but a statistically significant majority of subjects will follow a common response to a particular physiological fault.

Having confirmed that the injury was "reproducible," it was considered possible that after such an injury, the residual degree of muscle pathology or phase of healing, by inference, would inform us of the "severity" of the contusion at the time of injury. To test whether the injury was reproducible and measurable estimation of the severity of the injury was made from the macro- and microscopic appearance of the muscle using a simple scoring system.

Our initial preconception was that the injury would be greatest at the point of impact beneath the skin, reducing as the distance from impact decreased. This is patently not the case. Our results confirm that in this model there are at least two degrees of injury, one at the point of impact and second deep injury when the force causing the contusion essentially "crushes" the muscle between the skin and bone. The scores derived from the morphological appearance of the tissue show that while the muscle in both SD and JB positions exhibit evidence of trauma, the muscle closest to the bone exhibits the most damage. Here the muscle fibres and capillaries are crushed, causing myofibre necrosis capillary damage, local haemorrhage and connective tissue destruction. Such damage requires the phagocytic removal of necrotic tissue and replacement of myofibres, capillaries and interfibre connective tissue (endomysium and perimysium). A contusion injury, therefore, causes a complex, position dependent injury with consequent processes of muscle regeneration and repair. The simple scoring method employed in this study based on morphological doesn't enable severity of the injury to be adequately quantified- largely as consequence of the dearth of quantifiable numeric data. However, if this can be improved, the severity of injury expressed as morphological numeric score may be useful as a means of measuring and comparing the severity of an injury and may be used as a means of assessing the efficacy of a treatment regimen. The confirmation of the presence of two injuries of differing severity from a single contusion may be advantageous to physiotherapists when comparing the benefits of different treatment modalities.

5. Ethical Consideration

This study was approved by the Ethics and Research Committees of the University of KwaZulu-Natal on a yearly basis to accommodate any changes that might have occurred in the process of research project using animals from 2009, References: 022/09/Animal; 018/10/Animal; 022/11/Animal; 010/12/Animal; and 069/13/Animal.

6. Conclusion

These data, therefore describe two injuries of different severity following contusion in this model. Immediately beneath the area of impact, there is a significant injury to myofibres and capillaries sometimes causing bleeding. The most severe damage occurs where the muscle is compressed against the dense bone. Differences in the severity of the injury expressed as a morphological score suggest that this methodology, with refinement, maybe be employed to quantify the efficacy of a physiotherapeutic modality in assisting the healing of muscle trauma.

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References

[1] M. Deane, M. A. Gregory, and M. Mars, Histological and morphometric changes in untraumatised rabbit skeletal muscle treated with deep transverse friction, *South African Journal of Physiotherapy*, **58**, 28–33, (2002).

[2] M. A. Gregory, M. Deane, and M. Mars, Ultrastructural changes in untraumatised rabbit skeletal muscle treated with deep transverse friction, *Physiotherapy*, **89**, 408–416, (2003).

[3] M. A. Gregory and M. Mars, The effect of compressed air massage on untraumatised rabbit skeletal muscle – a morphometric and ultrastructural study, *South African Journal of Physiotherapy*, **60**, 19–27, (2004).

[4] M. A. Gregory and M. Mars, Compressed air massage causes capillary dilation in untraumatised skeletal muscle: a morphometric and ultrastructural study, *Physiotherapy*, **91**, 131–137, (2005).

[5] J. J. Crisco, P. Jokl, G. T. Heinen, M. D. Connell, and M. M. Panjabi, A muscle contusion injury model. Biomechanics, Physiology, and Histology, *American Journal of Sports Medicine*, **22**, 702–710, (1994).

[6] V. B. Minamoto, C. R. Grazziano, and T. F. Salvini, Effect of single and periodic contusion on the rat soleus muscle at different stages of regeneration, *Anatomical Record*, **254**, 281–287, (1999).

[7] J. R. Bunn, J. Canning, G. Burke, M. Mushipe, D. Marsh, and G. Li, Production of consistent crush lesions in murine quadriceps muscle - A biomechanical, histolomorphological and immunohistochemical study, *Journal of Orthopaedic Research*, **22**, 1336–1344, (2004).

[8] B. Fisher, V. Baracos, C. M. Hiller, and S. G. A. Rennie, A comparison of continuous ultrasound and pulsed ultrasound on soft tissue injury markers in the rat, *Journal of Physical Therapeutics Science*, **15**, 65–70, (2003).

[9] M. Deane, M. A. Gregory, and M. Mars, Creation of a contusion injury in rabbit skeletal muscle using a drop mass technique, *Journal of the South African Veterinary Association*, (2013), Under review.

[10] M. J. Karnovsky, A formaldehyde glutaradehyde fixative of high osmolarity for use in electron microscopy, *Journal of Cell Biology*, **27**, 127–139, (1965).

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