Can vasculature changes be characterised morphologically after closed soft tissue trauma?

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INTRODUCTION

Closed soft tissue trauma (CSTT) can be the result of a blunt impact, or a prolonged crush injury and involves damage to the skin, muscles and the neurovascular system. It causes a variety of symptoms such as haematoma and in severe cases may result in hypoxia and necrosis. There is evidence that early vasculature changes following the injury delays the tissue healing [1]. However, a precise qualitative and quantitative morphological assessment of vasculature changes after trauma and the effect of this on CSTT healing is currently missing.

Research aims: Developing an experimental rat model to characterise the structural changes to the vasculature after trauma qualitatively and quantitatively using micro CT.

MATERIAL AND METHODS

An impact device was developed to apply a controlled reproducible CSTT to the left thigh (Biceps Femoris) of anaesthetised rats [3]. After euthanizing the animals at 6 hours after trauma, CSTT was qualitatively evaluated by macroscopic observations of the skin and muscles. For vasculature visualisation, the blood vessels of sacrificed rats were flushed with heparinised saline and then perfused with a radio-opaque contrast agent (Microfil) using an infusion pump (Figure 4). The overall changes to the vasculature as a result of impact trauma were characterised qualitatively based on the 3D reconstructed images of the vasculature (Figure 5). For a smaller region of interest, the morphological parameters such as vessel thickness (diameter), spacing, and average number per volume were quantified using the scanner's software.

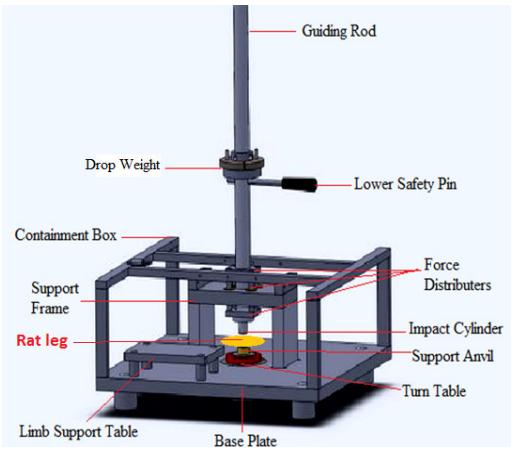
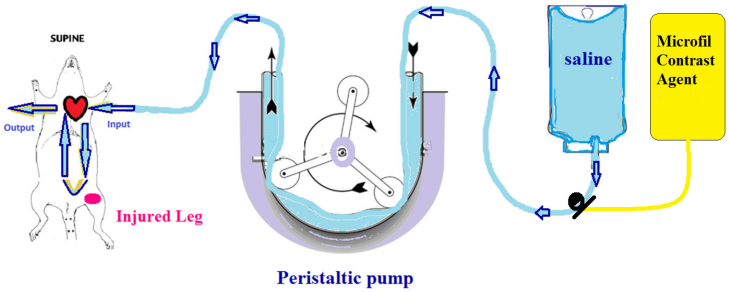


Figure 1: Drawing of the impact device for creating the CSTT and vascular injury on the defined region of rat leg (Biceps Femoris muscle on top and Gracillis on bottom) with impact weight of 181.4 g and an impact velocity of 5.1 m/s.

Figure 2: (below) Schematic of the perfusion protocol. After flushing of the vessels with heparinised saline, the contrast agent (Microfil, Flowtech, USA) was perfused.



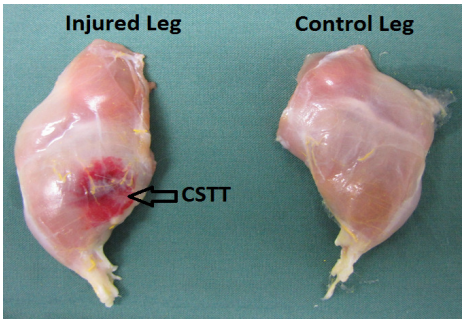


Figure 3: A view of the lateral surface of the injured and control legs is shown. The circular shape of the haematoma caused by the CSTT with a diameter of 1.5 cm on the Biceps Femoris can be seen.

RESULTS AND DISCUSSION

Visual observation of CSTT has revealed a haematoma in some animals (Figure 3). Micro CT images indicate good perfusion of the vasculature with contrast agent, allowing the major vessels to be identified (Figure 5). Qualitatively and quantitatively, no differences between injured and non-injured legs were observed at 6 h after trauma. Further time points of 12h, 24h, 3 days and 14 days after trauma will be characterised for identifying temporal changes of the vasculature during healing. Histomorphometical studies are required for validation of the results derived from the micro CT imaging.

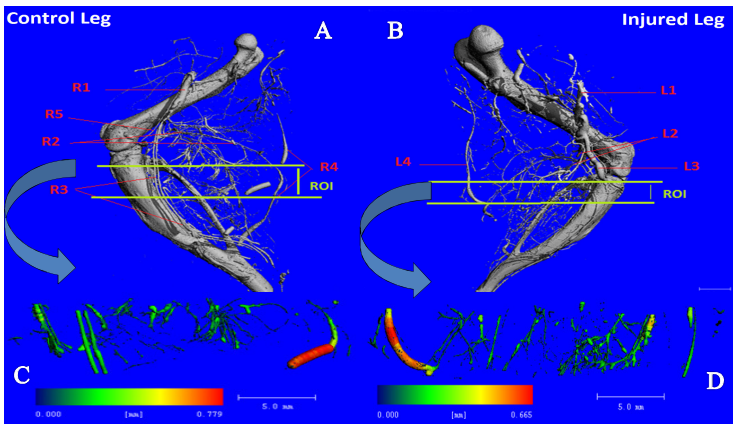


Figure 4: The vasculature from 3D reconstructions of the control (A) and the injured (B) dissected legs with micro CT (µCT 40, Scanco Medical, Switzerland) with energy and intensity of 55 kVp and 145 µA respectively The major arteries recognised for both right (R) and left (L) legs are: *1*_arteria femoralis, *2*_arteria saphena, *3*_arteria poplitea, *4*_arteria caudalis femoris distalis, *5*_arteria genus descendens. The figures C and D show the calculated vessel diameters using a colour coded scale for the ROI (30% of the distance between knee joint and tibia-fibula conjunction). The vessel diameter was quantified using a direct distance transformation technique.

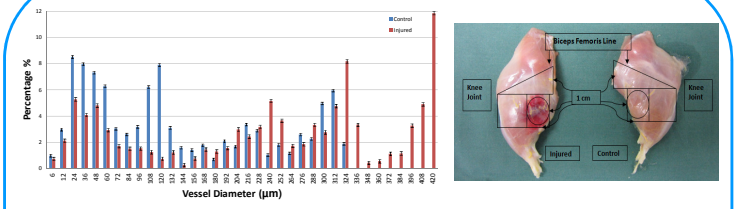
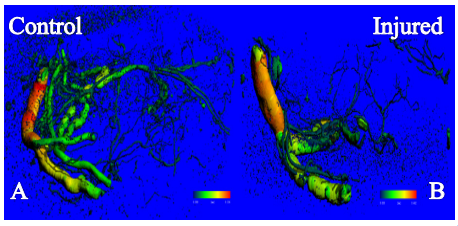


Figure 5: Full thickness biopsies were taken from the region of soft tissue trauma of control and injured legs and micro CT scanned. A histogram of all diameters of blood vessels indicates a reduction of small diameter vessels 6h after CSTT.



CONCLUSION AND FUTURE DIRECTION

Findings of this research may contribute towards the establishment of a fundamental basis for the quantitative assessment and monitoring of CSTT based on microvasculature changes after trauma, which will ultimately allow for optimising the clinical treatment and improve patient outcomes.

ACKNOWLEDGEMENT

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