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## THE EFFECT OF CRYOTHERAPY ON THE VASCULAR REGENERATION FOLLOWING CLOSED SOFT TISSUE TRAUMA

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### INTRODUCTION

Icing (cryotherapy) is being widely used for the treatment of closed soft tissue trauma (CSTT), such as those resulting from sport injuries. It is believed that cryotherapy induces vasoconstriction and through this mechanism reduces inflammation [1]. However, the impact of this technique on the healing of impaired vasculature and muscle injuries following trauma remains controversial. Recent evidence suggests that the muscle regeneration is delayed after cryotherapy [2]. Consequently, we aimed to investigate the effect of cryotherapy on the vascular morphology following CSTT using an experimental model in rats by contrast-enhanced micro-CT imaging.

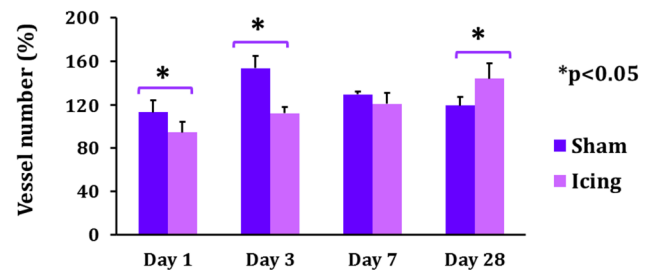
### METHODS

Fifty four rats were divided into three main groups: control (no injury, n=6), sham (CSTT but no icing treatment, n=24) and icing (CSTT, treated with one session of ice block massaged directly on the injured muscle for 20 minutes, n=24). The CSTT was induced to the left thigh (Biceps Femoris) of anaesthetised rats (Male, Wistar) to create a standardized and reproducible vascular and muscle injury using an impact device [3]. Following trauma, animals were euthanized after 1, 3, 7, and 28 days healing time (n=6 for each time point). For a three-dimensional vascular morphological assessment, the blood vessels of euthanised rats were flushed with heparinised saline and then perfused with a radio-opaque contrast agent (Microfil, MV 122, Flowtech, USA) using an infusion pump. Both hind-limbs were dissected, and then the injured and non-injured limbs were imaged using a micro-CT scanner ( $\mu$ CT 40, Scanco Medical, Switzerland) and total volume of the perfused blood vessels (TVV) was calculated. More detailed morphological parameters such as vessel volume (VV), diameter (VD), spacing (VSp), number (VN) and connectivity (VConn) were quantified through high resolution (6  $\mu$ m), micro-CT-scanned biopsy samples (diameter: 8mm) taken directly from the region of the injured muscles. The biopsies were then analysed histologically to confirm the results derived from contrast-enhanced micro-CT imaging.

### RESULTS AND DISCUSSION

The TVV was significantly higher in the injured legs compared to the non-injured legs at day 1 and 7 in the sham group and at day 28 in both sham and icing groups. The biopsies from the injured legs of the icing group showed a

significant reduction in VV, VN, VD, VConn and an increase in VSp compared to those in the sham and control groups at days 1, 3 and 7, post injury. While the injured legs of the sham group exhibited a decrease in VN and VConn 28 days post trauma, indicating a return to the original values prior to trauma, these parameters had increased in the icing group (Figure 1). Also, at day 1 post injury, VV and VD of the injured legs were significantly higher in the sham group compared to the icing group, which may be attributed to the effect of vasoconstriction induced by icing. Further histomorphological evaluation of day 1 post injury, indicated that although cryotherapy significantly reduced the injury size and influx of inflammatory cells, including macrophages and neutrophils, a delay in vascular and muscle fiber regeneration was found at later time points confirming other reports from the literature [2].



**Figure 1:** The average number of vessels per volume of the biopsies from the injured legs normalized to the contralateral non-injured legs (Mean $\pm$ SD), indicating higher values for the sham group at early time points, and higher values for the icing group at the latest time point.

### CONCLUSIONS

We have demonstrated using micro-CT imaging that the vascular morphology changes after CSTT, and that its recovery is affected by therapeutic modalities such as icing. This may be useful for the development of future clinical monitoring, diagnosis and treatment of CSTT. While icing reduces the swelling after trauma, our results suggest that it may delay the recovery of the vasculature in the injured tissue.

### REFERENCES

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