

## **Ischaemic Myotubes Stimulate Angiogenesis via Exosomes**

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### *Background*

Skeletal myotube exosomes are important in the repair and regeneration of healthy muscle however, we have shown that myotubes in an ischaemic microenvironment will secrete exosomes that impair muscle differentiation, potentially contributing to muscle dysfunction in peripheral arterial disease (PAD). As PAD is a systemic vascular disease we now investigate the role of exosomes in the crosstalk between muscle and endothelial cells and the impact of ischaemic exosomes on angiogenesis.

### *Methods*

Exosomes were isolated from C2C12 myoblasts (MB), myotubes (MT) and ischaemic myotubes (IMT) through ultracentrifugation. Presence of exosomes was confirmed using Nanosight, western blotting and electron microscopy (EM). PKH26 labelled exosomes were co-cultured with human umbilical vein endothelial cells (HUVECs) for 12 and 24hrs. Exosome uptake was assessed by fluorescent microscopy and FACs. The functional effect of IMT-exosomes on HUVECs was assessed through angiogenesis assays.

### *Results*

Nanosight data confirmed exosome populations of similar size and frequency secreted by MTs and IMTs (MT-exosome peak 107nm, concentration  $4.85e+008 \pm 2.74e+007$  particles/ml IMT-exosome peak 98nm, concentration  $6.08e+008 \pm 1.68e+007$  particles/ml). EM and western blotting showed that MT- and IMT-exosomes are similar in structure and positive for known exosomal markers CD81 and Alix. Co-culture of 25ul exosomes with HUVECs resulted in uptake of MB- MT- and IMT- exosomes over 24hrs. Angiogenesis assay demonstrated that IMT-exosomes stimulate tube formation in a concentration dependent manner.

### *Conclusion*

Our data demonstrates that muscle exosomes are readily taken up by HUVECs, suggesting a novel method of muscle- vasculature intercellular communication. An ischaemic microenvironment alters the exosomal message, which in turn stimulates angiogenesis. In ischaemia, clarifying the role of exosomes in angiogenesis may provide novel targets that can be manipulated for patient benefit.