

## Hind limb ischemia in type 1 diabetic mice as useful tool to evaluate the neovascularization of tissue engineering constructs

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Hind-limb ischemia has been used in type 1 diabetic mice to evaluate treatments for peripheral arterial disease or mechanisms of vascular impairment in diabetes [1]. Vascular deficiency is not only a pathophysiological condition, but also an obvious circumstance in tissue regeneration and in tissue engineering and regenerative medicine (TERM) strategies.

We performed a pilot experiment of hind-limb ischemia in streptozotocin(STZ)-induced type 1 diabetic mice to hypothesise whether diabetes influences neovascularization induced by biomaterials. The dependent variables included blood flow and markers of arteriogenesis and angiogenesis.

Type 1 diabetes was induced in 8-week-old C57BL/6 mice by an i.p. injection of STZ (50 mg/kg daily for 5 days). Hind-limb ischemia was created under deep anaesthesia and the left femoral artery and vein were isolated, ligated, and excised. The contralateral hind limb served as an internal control within each mouse. Non-diabetic ischaemic mice were used as experiment controls. At the hind-limb ischemia surgical procedure, different types of biomaterials were placed in the blood vessels gap. Blood flow was estimated by Laser Doppler perfusion imager, right after surgery and then weekly. After 28 days of implantation, surrounding muscle was excised and evaluated by histological analysis for arteriogenesis and angiogenesis.

The results showed that implanted biomaterials were promote faster restoration of blood flow in the ischemic limbs and improved neovascularization in the diabetic mice. Therefore, we herein demonstrate that the combined model of hind-limb ischemia in type 1 diabetes mice is suitable to evaluate the neovascularization potential of biomaterials and eventually tissue engineering constructs.

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### References:

1. Dokun AO, Chen L, Lanjewar SS, Lye RJ, Annex BH (2014) Glycaemic control improves perfusion recovery and VEGFR2 protein expression in diabetic mice following experimental PAD. *Cardiovasc Res* 101: 364-372.