Optimized Murine Hindlimb Ischemia Model to Assess Vascular Regeneration in Peripheral Artery Disease

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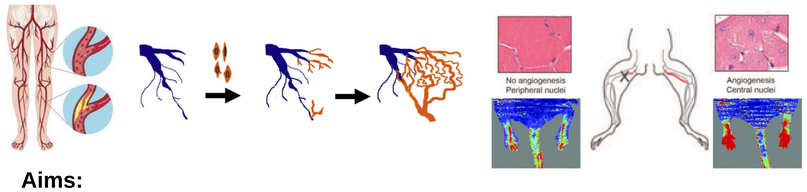
Introduction

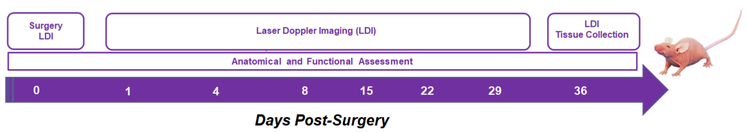
- Lower extremity peripheral arterial disease (PAD), affects 236 million patients worldwide. Ischemia effects in patient's musculature results in reduced muscle area, increased fatty infiltration and fibrosis, and metabolic and cellular abnormalities, leading to impaired walking performance, loss of mobility and, in severe cases, limb amputations.[1]
- Transplantation of autologous cells can potentially regenerate vascular tissues and restore blood flow. [2]
- The murine acute hindlimb ischemia model is commonly used to evaluate these therapeutics. However, inconsistent outcomes in clinical trials, derived from data obtained with this model, may indicate limitations in its ability to accurately reflect the clinical condition of PAD, hindering therapeutic development.[3]



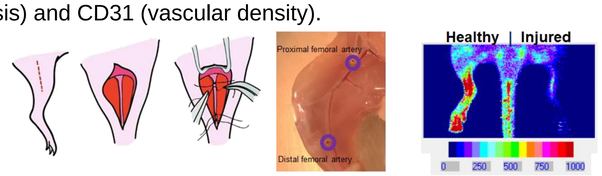
Aims:

1) To optimize the PAD murine hindlimb ischemia model, by examining the impact of variables such as age and sex on tissue regeneration, by evaluating the vascular and muscular characteristics in the model.
2) To improve the translational potential of rodent findings to preclinical evaluations of cell-based regenerative therapies.

Methods

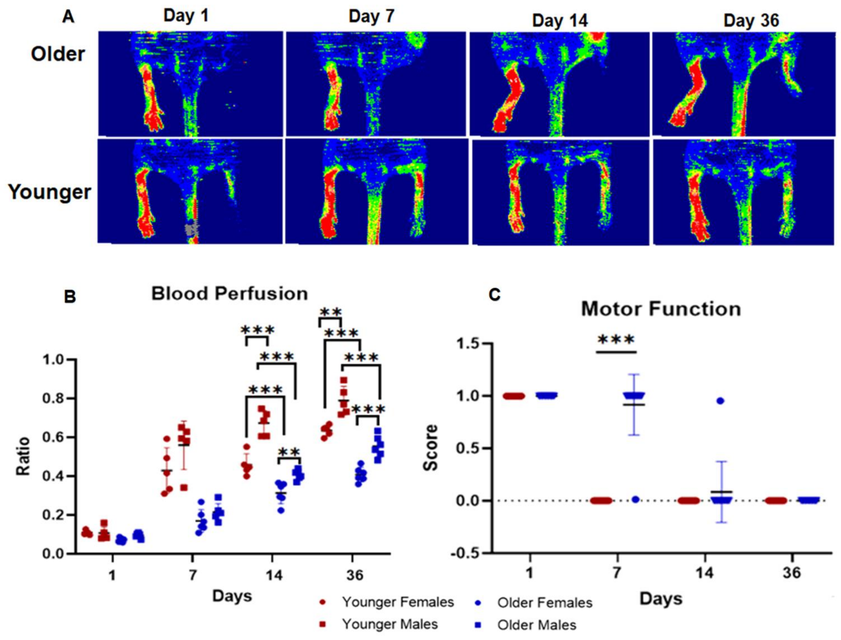


Animals: Male and female nude mice (NU/NU), 6.5 and 12.5 weeks old (n=6)
Surgical procedure: Double-knotted ligations on the right femoral artery to induce ischemia (approved by NU- IACUC)
Imaging: Laser Doppler Imaging (LDI) to measure blood perfusion on healthy and injured limbs.
Functional Assessment Score: 0 for normal walking, 1 for limping, or 2 for dragging the limb
Anatomical Assessment Score: 0 for no apparent damage, 1 for evident necrosis on nails, 2 for necrosis on digits and 3 if the damaged tissue reached the paw area
Histological Assessment: Masson's Trichrome (average muscle fiber cross sectional area, percentage of centralized nuclei, infiltration of adipose-like tissue and fibrosis) and CD31 (vascular density).

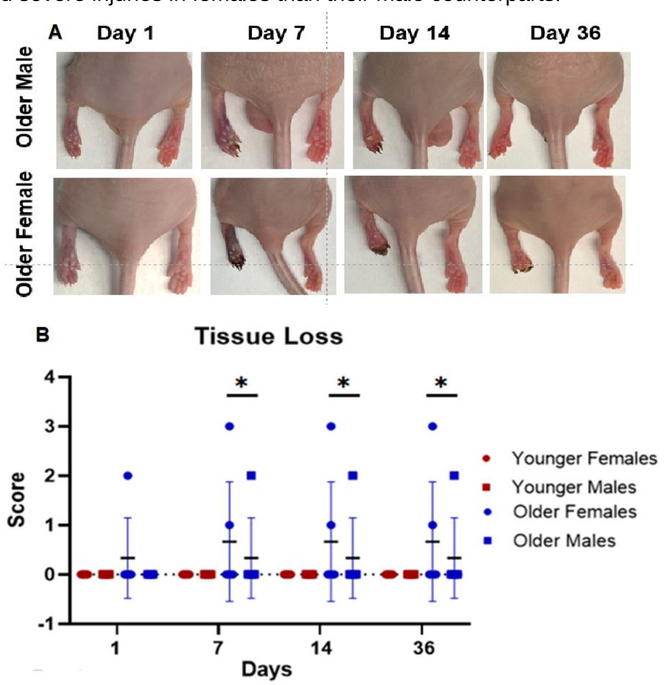


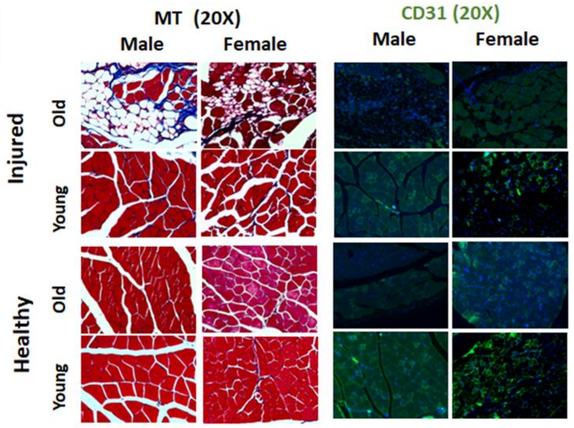
Results

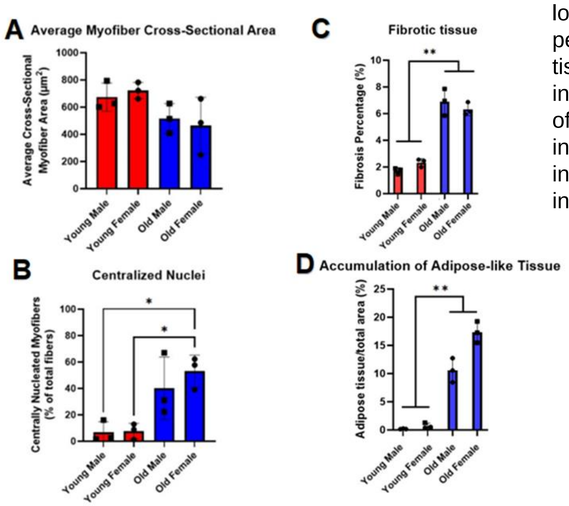
(**1**) There were significant differences in blood perfusion rates among all experimental groups, from week two until the end of the observational period (**A, B**). Motor function (**C**) was equally impaired among groups following surgery; young mice returned to normal walking within one week, while the older groups took an additional week to regain normal walking motion.



(**2**) The anatomical assessments showed that none of the young mice exhibited necrosis (**B**), while the older cohort (**A**) experienced more frequent and severe injuries in females than their male counterparts.







(**3**) Histological data showed an increase in muscle atrophy 36 days after the onset of ischemia in the older groups, when compared to the younger ones. Irregular myofiber shape (**A**), centralized nuclei location (**B**), higher percentage of fibrotic tissue (**C**) and an increased accumulation of adipose-like tissue (**D**) indicate worse outcomes in terms of regeneration in the older mice.

Conclusions

Functional and anatomical findings align with clinical observations, indicating that female PAD patients tend to experience more complications than males.

Histological findings are consistent with calf muscle pathology in human PAD patients.

Age and sex of mice influence the functional, anatomical and histological outcomes of the murine PAD model.

IMPACT: our findings suggest that age and sex should be taken into consideration when evaluating the effectiveness of regenerative therapies for limb salvage and long-term vascular regeneration in PAD patients.

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References

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