communication involving an array of cell types. These interactions are not well-understood, and the development of novel regenerative therapies for peripheral arterial disease will require new insight into the cellular heterogeneity and intercellular signaling that occurs in the ischemic limb. Macrophages play a role in orchestrating critical events in muscle regeneration; hence we sought to characterize their transcriptional signature and identify candidate signaling pathways in the context of limb ischemia.

**Methods:** We applied single-cell RNA sequencing to skeletal muscle obtained from regenerative (C57BL/6) and nonregenerative (BALB/c) mouse limbs at multiple time points following ligation of the femoral artery. We used CellChat, a computation tool, to predict signaling pathway activity between macrophages and muscle satellite cells (MuSCs) based on our single-cell data.

**Results:** We identified 12 distinct macrophage populations in the regenerative and nonregenerative limb, including strain-specific macrophage clusters and MuSCs. Notable potential interactions included enhanced FNT1 to SDAC and THBS1 to SDC4 signaling between regenerative macrophage and MuSCs, as well as OPN to CD44 signaling between nonregenerative macrophages and MuSCs.

**Conclusions:** Our study maps the dynamic macrophage response in the ischemic limb at single-cell resolution. This provides a valuable resource to investigate macrophage-mediated mechanisms of skeletal muscle regeneration in response to limb ischemia.

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**Abstracts**

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**Distinct Satellite Cell Trajectories Characterize Regenerative and Nonregenerative Responses in the Ischemic Limb**

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**Background:** Skeletal muscle regeneration is a critical determinant of outcomes in the ischemic limb. The precise mechanisms of skeletal muscle recovery in the ischemic limb remain unknown. A major hindrance to understanding the regenerative process in limb ischemia is determining the heterogeneity and cell fate decisions of muscle satellite cells (MuSCs).

**Methods:** We analyzed muscle regeneration in C57BL/6 (regenerative phenotype) and BALB/c (nonregenerative phenotype) mice undergoing limb ischemia using single-cell RNA sequencing spanning four distinct time points (no injury, days 1, 3, and 7). We also performed trajectory inference to observe stage-specific regulatory programs during this dynamic process.

**Results:** We identified nine distinct MuSC populations in both strains (Figure A). Pseudotime analysis presented an organized regeneration trajectory of cells from quiescent to proliferative to differentiated muscle stem cells (MuSCs) (Figure B). Further analysis demonstrated disparate MuSC fate decisions between C57BL/6 and BALB/c mice (Figure D). C57BL/6 mice displayed an organized progression of cells from a quiescent state to proliferative and committed myoblasts, while BALB/c mice demonstrated aberrant MuSC regeneration, highlighted by precocious differentiation (Figure F). Furthermore, gene set enrichment analysis confirmed impaired MuSC proliferation in BALB/c mice (Figure F).

**Conclusions:** These findings advance our understanding of MuSC fate decisions in the ischemic limb and provide a potential mechanism for myopathy observed in patients with peripheral artery disease.