Aging-Induced Discrepant Response of Fracture Healing at the Multiple Scales

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Summary

Collagen network and minerals are the fundamental components to restores the structural and mechanical integrity of bone during fracture healing. Aging has been well recognized to affect bone quality. However, how the structural and mechanical recovery of fracture healing is influenced by aging, particularly from the perspective of organization and mineralization of the collagen network, remains unclear. A tibial fracture model was established for both the young and aged mice. The characteristics of collagen network, mineralization and nanoscale mechanical properties, of the callus were assessed. The results indicated that aging-induced structure and mechanical differences of callus during fracture healing initiate from the organization and mineralization of collagen fibrils. Multi-scale structural and mechanical analysis suggests mechanical immobilization is beneficial to the structure, composition, and mechanics of callus in the aged mice, while impairing the organization and mineralization of collagen fibril in callus of the young mice.

Introduction

Fracture healing is a complex and well-orchestrated process involving callus formation and bone remodeling. Callus mineralization is affected by mechanical environment during healing. In the present study, how mechanical environment affect the fracture healing and quality of bone callus of mice at different age was investigated.

Methods

A murine tibial fracture model was established by osteotomies. All mice in the young group and aged group (5 weeks *v.s.* 68 weeks) were anesthetized by inhaling isoflurane during the operation. A classical internal fixation approach with a steel needle inserted into the tibia marrow cavity was adopted for osteosynthesis. The mechanical immobilization intervention was achieved by using a hind limb tail suspension approach. Following the fracture operation, mice in the young and aged groups were randomly separated into a control group, *i.e.*, young-control and aged-control group, and an immobilization group, *i.e.*, young-immobilization and aged-immobilization group, respectively. The mice in the control group could move freely, while the mice in the immobilization group underwent tail suspension during the experiment.

On the 21st day postoperation, the left tibia was harvested. Microstructure, multiplescale mechanical properties, composition of callus were assessed using wide-angle X-ray diffraction, micro-CT (μ CT), Backscatter electron (BSE) imaging combined energy dispersive spectrometer (EDS), Raman Spectroscopy, nanoindentation and 3-point bending tests.

Results and Discussion

The results indicated that aging postpones the fracture healing process, leading to incomplete microstructure, less mineral content and mineralization, and weaker mechanical properties of callus. In the aged mice, the internal fixation and mechanical immobilization promoted the mineralization of callus by increasing mineral crystal length and mineral-to-matrix ratio by 48% and 42% comparing to the internal fixation and free movement control group, respectively. By contrast, in the young mice, the internal fixation and mechanical immobilization induced disordered collagen fibrils, decreased the crystal length and mineral-to-matrix ratio by 32% and 36%, comparing to the internal fixation and free movement control group, respectively.

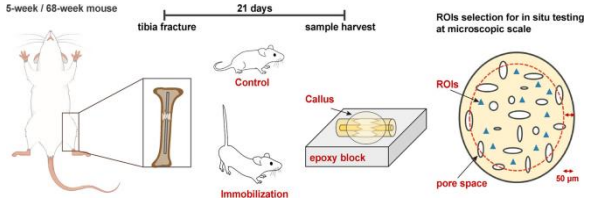

Figure 1: Experimental setup of the present study.

Conclusions

The present findings suggested that aging-induced structure and mechanical differences of callus during fracture healing initiate from the organization and mineralization of collagen fibrils. Multi-scale structural and mechanical analysis suggests mechanical immobilization is beneficial to the structure, composition, and mechanics of callus in the aged mice, while impairing the organization and mineralization of collagen fibril in callus of the young mice. These findings suggested that different mechanical intervention strategies should be adopted for the fracture healing at different ages, which provides valuable insights for the clinical treatment of bone fracture.

Acknowledgments

The present work was supported by the National Natural Science Foundation of China (grant numbers 12272317 and 12302403).

References

[1] Liu F et al. (2025). *ACS Biomater Sci Eng*, **11**: 1038-50.
[2] Depalle B et al. (2021). *Acta Biomater*, **120**: 194– 202.
[3] Liu F et al. (2022). *Acta Biomater*, **152**: 345– 354.