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Research paper

Hierarchies of damage induced loss of mechanical properties in calcified bone after *in vivo* fatigue loading of rat ulnae

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ABSTRACT

During fatigue loading of whole bone, damage to bone tissue accumulates, coalesces and leads to fractures. Whether damage affects tissue material properties similarly at the nanoscale (less than 1 μm), microscale (less than 1 mm), and whole bone scale has not been fully evaluated. Therefore, in this study, we examine scale-dependent loss of calcified tissue material properties in rat ulnae, after fatigue loading of rat forearms using the forearm compression model. *In vivo* fatigue loading was conducted on the right forearms until a displacement end-point was reached. The non-fatigued left forearms served as contralateral controls. Subsequently, three-point bending tests to failure on excised ulnae demonstrated a 41% and 49% reduction in the stiffness and ultimate strength as compared to contralateral control ulnae, respectively. Depth-sensing microindentation demonstrated an average decrease in material properties, such as elastic modulus and hardness, of 28% and 29% respectively. Nanoindentation measured elastic modulus and hardness were reduced by 26% and 29% in damaged bone relative to contralateral controls, respectively. The increased loss of whole bone material properties compared to tissue material properties measured using indentation is mainly attributed to the presence of a macrocrack located in the medial compressive region at the site of peak strains. The similar magnitude of changes in material properties by microindentation and nanoindentation is attributed to damage that may originate at an even smaller scale, as inferred from 10% differences in connectivity of osteocyte canaliculi in damaged bone.

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