Bisphosphonate-Induced Remodeling Suppression Further Degrades Material Properties in Fatigue-Damaged Bone

Niovi T Dollas[1], Abigail A Coffman[1], Lukasz Witek[2,3,4], Mitchell B Schaffler[1]
[1]Dept of Biomedical Engineering, The City College of New York, New York, NY, [2]Biomaterials Division, NYU College of Dentistry [3]Dept of Biomedical Engineering, NYU Tandon School of Engineering and [4]Hansjörg Wyss Dept of Plastic Surgery, NYU Grossman School of Medicine, New York, NY
ndollas000@citymail.cuny.edu

Disclosures: Niovi Dollas (N), Abigail Coffman (N), Lukasz Witek (N), Mitchell Schaffler (N)

INTRODUCTION: Long-term use of bisphosphonates (BP) results in the accumulation of microcracks (μCrks) in bone. [1] Such μCRks are implicated in the pathogenesis of atypical femoral fractures, though the precise nature of this interaction is not well understood. [2] There is a fundamental question as to what happens to the small amount of μCrks and the associated impairments of local material properties in remodeling-suppressed living bone, especially with normal low-level mechanical loading, especially if those cracks cannot be remodeled as in BP treatment. In the current studies, we tested whether small amounts of "pre-existing" fatigue microdamage will increase and local material properties will degrade during BP treatment with normal, *in vivo* loading (i.e., cage ambulation).

METHODS: Bone microdamage induction and treatment- Ulnae of young-adult (4 m.o.) female Sprague Dawley rats (n=24) were cyclically loaded in end-load bending *in vivo* to induce controlled amounts of fatigue (FAT) microcracks (μCrks) in the mid-diaphyseal cortex, as previously described. [3] Control ulnae were not subjected to fatigue loading (NoFAT). Baseline animals were sacrificed immediately following damage induction; Survival animals were treated for 4 months following damage induction with either alendronate (ALN) or PBS, after which point they were sacrificed. Accordingly, experimental groups are Baseline-NoFAT, Baseline-FAT, ALN-NoFAT, ALN-FAT, PBS-NoFAT, PBS-FAT. Microcrack content- Ulnae were stained *en bloc* with basic fuchsin to identify μCrks. After PMMA embedding, 200 μm-thick cross sections were prepared from the damage region of FAT ulnar diaphyses, or the equivalent location in NoFAT ulnae, and cover-slipped for microscopy; microcrack density (Cr.Dn, #/mm^2) was measured using OsteoMeasure and fluorescence microscopy. Mechanical properties: Additional ulnar diaphyseal cross sections were prepared for nanoindentation to measure local elastic modulus (E, GPa). Indentation testing was performed using a Hysitron TI-950 TriboIndenter. Tests were conducted using a 5,000 μN maximum load, 10 sec loading/unloading time and 20 sec hold-time, with indents placed 10 μm apart. Modulus was measured for: 1) Overall bone (i.e., all bone) in the microdamage containing cortical region in these ulnae, and 2) in bone immediately surrounding (±30 μm) μCrks (i.e., around dx) in Baseline-FAT and ALN-FAT bones. Note, PBS-FAT samples had already undergone osteonal remodeling of the microdamage by 4 months, removing most of the μCrks (Fig 2). In these bones, local measurements were made on osteons (i.e., remodeled area) instead. Statistics- Differences in Cr.Dn and moduli were assessed using a one-way ANOVA with multiple comparison post-hoc testing (GraphPad Prism) and data are reported as mean ± SD.

RESULTS: Mechanical Properties: Loss of modulus in baseline fatigue ulnae (Baseline-FAT) was highly localized, with E around μCrks reduced ~20% vs. NoFAT bone; there was no reduction is overall tissue modulus beyond the bone around μCrks. In contrast, ALN-treatment in fatigued bone caused a marked decrease in overall modulus (~25% vs ALN-NoFAT), with an even greater decline of local modulus loss in the bone surrounding microdamage. Overall E of PBS-FAT bone was similar to control, but E of osteons was ~20% lower than control bone. Tissue modulus data are summarized in Fig 1. Microcrack content: Cr.Dn was increased ~60% in ALN-treated fatigued bone vs Baseline-FAT bone. In contrast, in PBS-FAT bone, where remodeling occurred, μCrk content was reduced by almost 75% vs Baseline-FAT bone. Cr.Dn data are summarized in Fig. 2.

DISCUSSION: The current studies show that suppression of bone remodeling in previously fatigued bone has a marked negative impact, with ALN use resulting in both increased number of microcracks and expanded degradation of local mechanical properties in bone. The mechanisms by which ALN treatment of bone with pre-existing microdamage further degrades material properties are not yet fully understood. However, it is well established that fatigue microcracks cause locally impaired bone stiffness. [3-5] Accordingly, continued loading of bone foci with unremodeled microcracks will be expected to result in locally elevated stresses, which, in turn, can mechanically drive formation of additional microcracks. Other potential contributors to matrix degradation and microcrack accumulation include osteocyte effects such as localized osteocyte death known to occur around microcracks and also the metabolic stress in surviving osteocytes near microcracks- both of which can potentially make bone more susceptible to matrix damage. [3, 6-9]

SIGNIFICANCE: The present study shows that if cortical bone remodeling cannot occur due to bisphosphonate treatment, small amounts of experimentally-placed bone microdamage *in vivo* can readily increase and markedly impair local material properties, even with normal low-level mechanical loads.

REFERENCES: 1) Allen+ Bone 2006; 2) Starr+ Curr Osteopor Rep 2018; 3) Bentolila+ Bone 1998; 4) Boyce+ JOR 1998; 5) Seref-Ferlengez BoneKEy Rep 2015; 6) Verborgt+ JBMR 2000; 7) Tami+ JBMR 2002; 8) Knothe Tate+ Trans ORS 2002; 9) Schaffler+ Calcif Tissue Int 2013

ACKNOWLEDGEMENTS : AR081381, AR041210 and AR070547 from NIAMS; AG056397 from NIA

Fig. 1: Bone moduli for experimental groups

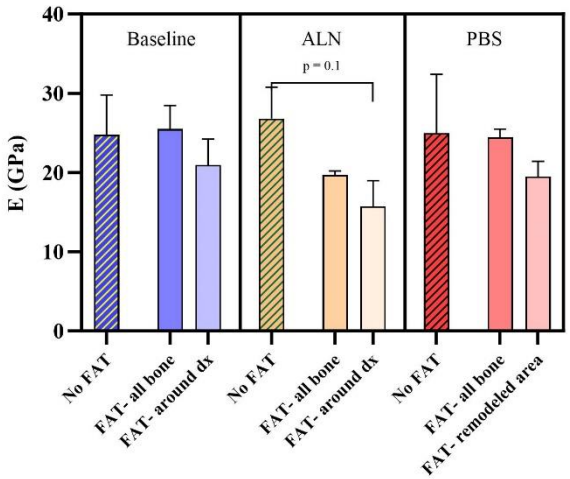

Fig. 2: Microcrack density for experimental groups