JOURNAL OF THE MECHANICAL BEHAVIOR OF BIOMEDICAL MATERIALS 48 (2015) 220–228

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jmbbm

Research Paper

Microarchitectural and mechanical characterization of the sickle bone

Mykel Green[a], *Idowu Akinsami*[b], *Angela Lin*[c], *Shereka Banton*[b], *Samit Ghosh*[d], *Binbin Chen*[b], *Manu Platt*[b], *Ifeyinwa Osunkwo*[e], *Solomon Ofori-Acquah*[d], *Robert Guldberg*[c], *Gilda Barabino*[a,b,*]

[a]*Department of Biomedical Engineering, The City College of New York, New York, NY 10031, USA*
[b]*Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[c]*Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332, USA*
[d]*Vascular Medicine Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA*
[e]*Levine Cancer Institute, Carolinas HealthCare System, Charlotte, NC 28204, USA*

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form
12 April 2015
Accepted 17 April 2015
Available online 24 April 2015

Keywords:
Sickle cell disease
Microarchitecture
Biomechanics
Micro-CT
Bone

ABSTRACT

Individuals with sickle cell disease often experience acute and chronic bone pain due to occlusive events within the tissue vasculature that result in ischemia, necrosis, and organ degeneration. Macroscopically, sickle bone is identified in clinical radiographs by its reduced mineral density, widening of the marrow cavity, and thinning of the cortical bone due to the elevated erythroid hyperplasia accompanying the disease. However, the microstructural architecture of sickle bone and its role in mechanical functionality is largely unknown. This study utilized micro-CT and biomechanical testing to determine the relationship between the bone morphology, tissue mineral density, and trabecular and cortical microarchitecture of 10- and 21-week-old femurs from transgenic sickle male mice and littermates with sickle trait, as well as a wild-type control. While bone tissue mineral density did not vary among the genotypes at either age, variation in bone microstructure were observed. At 10 weeks, healthy and trait mice exhibited similar morphology within the cortical and trabecular bone, while sickle mice exhibited highly connected trabeculae. Within older femurs, sickle and trait specimens displayed significantly fewer trabeculae, and the remaining trabeculae had a more deteriorated geometry based on the structure model index. Thinning of the cortical region in sickle femurs contributed to the displayed flexibility with a significantly lower elastic modulus than the controls at both 10- and 21-weeks old. Wild-type and trait femurs generally demonstrated similar mechanical properties; however, trait femurs had a significantly higher modulus than sickle and wild-type

Abbreviations: SCD, sickle cell disease; SS, homozygous recessive sickle cell disease; AS, heterozygous sickle trait; AA, wild-type control

*Corresponding author at: Department of Biomedical Engineering, The City College of New York, 160 Convent Ave, New York, NY 10031, USA.
E-mail address: gbarabino@ccny.cuny.edu (G. Barabino).

http://dx.doi.org/10.1016/j.jmbbm.2015.04.019

control at 21-weeks. Overall, these data indicate that the progressive damage to the microvasculature caused by sickle cell disease, results in deleterious structural changes in the bone tissue's microarchitecture and mechanics.

1. Introduction

Microvascular vaso-occlusion experienced by individuals living with sickle cell disease (SCD) is one of the most common reasons for hospitalization (Almedia and Roberts, 2005; Ejindu et al., 2007). These painful crises spontaneously manifest throughout the patient's life and are most frequently reported to be localized in the bone (Chiang and Frenette, 2005). Under normal circumstances, red bone marrow, the site of hematopoiesis, is temporarily present throughout the skeleton until it is replaced by yellow, or fatty, marrow in the peripheral bones. However, sickle patients exhibit erythropoietic hyperplasia resulting in the maintenance of and reconversion to red marrow (Mankad et al., 1990). Moreover, marrow infarctions due to crises occur more frequently in the red marrow than in yellow (Rao et al., 1989).

Infarcts within bone are considered clinically silent and tend to be discovered incidentally by conventional radiological scans, such as monochromatic X-rays (Ware et al., 1991). The hypoxic nature of the bone marrow microenvironment promotes erythrocyte sickling and necrosis (Smith, 1996). Collectively, infarcts and bone marrow expansion in the long bones of sickle patients contributes to cortical bone thinning and increasing of trabecular spacing, which may consequently expedite the deterioration of the bone's mechanical strength (Serjeant and Serjeant, 2001). Albeit an under investigated aspect of SCD, nearly 30% of adult sickle patients self-reported multiple fractures due to low impact trauma in various locations, including the vertebrae, wrist, metatarsal, and femoral bones (Arlet et al., 2013). Multiple fractures have also been reported in children with sickle cell disease as young as 19 months (Omer et al., 2013) and may continue to occur throughout adolescents, sometimes accompanied with osteomyelitis (Ebong, 1986).

Phenotypically, these observed changes in sickle bone closely resemble osteoporotic bone, from which it became standard clinical practice to utilize bone mineral density (BMD) levels obtained from dual-energy X-ray absorptiometry (DXA) to monitor bone health and predict fracture risk (Brinker et al., 1998; World Health Organization, 1994). However, the unreliable interpretations of DXA results have led to numerous misdiagnoses due to overestimating and underestimating BMD levels in general (Gafni and Baron, 2004; Wren et al., 2005). Additionally, variations in the BMD of sickle patients are based on age, gender, and ethnicity, further minimizing the usefulness of BMD to monitor sickle bone by itself (Almedia and Roberts, 2005; Gupta et al., 2009; Lal et al., 2006; Sarrai et al., 2007).

The utilization of microcomputed tomography (micro-CT) provides a platform for the fabrication of high resolution 3D images of the bone microenvironment in a non-destructive and time-lapsed fashion, furthering the ability to quantitatively assess bone quality (Bouxsein et al., 2010). The unique microarchitecture of the trabecular regions influences whole bone strength, yielding various mechanical properties dependent on quality and organization of the bone (Gibson, 1985; Turner et al., 1990; Ulrich et al., 1997). Unlike DXA, micro-CT determines the tissue mineral density (TMD) of the mineralized bone tissue only, which directly influences clinical BMD measurements but negates the attenuation of soft tissue (muscle, bone marrow, etc.) that may complicate interpretations. Currently, high resolution clinical micro-CT technology is limited only to the extremity regions of the body; however the use of animal models with micro-CT serves as critical tool for investigating human pathology (Wang et al., 2005). In lieu of such technological advancements and established knowledge, the bone phenotype in SCD has yet to be characterized beyond monochromatic X-rays.

To further examine the effects of SCD in bone, we performed micro-CT image analyses to elucidate TMD and the microarchitecture of trabecular and cortical bone of femurs collected from transgenic SCD (SS) mice and littermates with sickle trait (AS), as well as mice expressing normal human hemoglobin (AA). Femurs were collected at 10 and 21 weeks of age for image analyses as well as biomechanical testing to determine the mechanical properties respective to femur morphology. Our data demonstrate that TMD does not differ significantly between these three groups of mice. However, we found that differences in microarchitecture contribute to the unique mechanical properties of sickle bone.

2. Materials and methods

2.1. Experimental animals

In order to characterize the sickle bone phenotype, we employed a Townes transgenic sickle mouse model and C57BL/6 wild-type controls (Ghosh et al., 2012; Ryan et al., 1997). Mice were selectively mated and categorized into either homozygous (SS) or heterozygous (AS) expression as determined by PCR. Analysis using micro-CT and biomechanical testing was conducted on femurs were harvested without chemical fixation from 10- and 21-week-old male mice and stored frozen at −20 °C wrapped in phosphate buffer saline soaked gauze until time of analysis ($n=3$–6/group). All protocols were IACUC approved by Emory University and Georgia Institute of Technology.

2.2. Microcomputed tomography

The trabecular and cortical bone morphologies of femurs were examined by micro-CT imaging (μCT 40, Scanco Medical, SUI), as previously reported (Robertson et al., 2006). Morphological and mineral density analyses were conducted on the femoral, cortical, and trabecular regions scanned at a voxel size of 12 μm