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Spatial relationships between bone formation and mechanical stress within cancellous bone

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ABSTRACT

Bone adapts to mechanical stimuli. While in vivo mechanical loading has been shown to increase the density of cancellous bone, theory suggests that the relationship between tissue stress/strain and subsequent bone formation occurs at the scale of individual trabeculae. Here we examine bone formation one week following mechanical stimulus. Three bouts of cyclic loading (300 cycles/day on 3 consecutive days) were applied to caudal vertebrae of female rats ($n=7$). Bone formation was determined using three-dimensional images of fluorescent markers of bone formation ($0.7 \times 0.7 \times 5.0\ \mu m^3$) and local tissue stress/strain was determined using high-resolution finite element models. Three days of mechanical stimuli resulted in an increase in mineralizing surface (loaded: $17.68 \pm 2.17\%$; control: $9.05 \pm 3.20\%$; mean $\pm$ SD) and an increase in the volume of bone formed (loaded: $7.09 \pm 1.97\%$; control: $1.44 \pm 0.50\%$). The number of bone formation sites was greater in loaded animals (650.71 ± 118.54) than pinned not loaded controls (310.71 ± 91.55), a difference that was explained by the number of formation sites at regions with large local tissue strain energy density (SED). In addition, the probability of observing bone formation was greater at locations of the microstructure experiencing greater SED, but did not exceed 32%, consistent with prior work. Our findings demonstrate that bone formation in the week following a short term mechanical stimulus occurs near regions of bone tissue experiencing high tissue SED, although the ability of finite element models to predict the locations of bone formation remains modest and further improvements may require accounting for additional factors such as osteocyte distribution or fluid flow.

1. Introduction

The idea that bone adapts to habitual mechanical stimulation originated over 150 years ago and was based on observations that the alignment of cancellous bone microarchitecture matches the orientation of principal stresses (see Cowin (2001) for a thorough review). The degree to which bone density and internal and external morphology is altered in response to mechanical stimuli is key to understanding the potential of biophysical stimuli to improve bone microstructure and is also useful for the interpretation of trabecular bone alignment in rare specimens from the fossil record (Ryan and Ketcham, 2002).

Experimental application of controlled mechanical loads to the bones of live animals has provided a wealth of information on bone functional adaptation (van der Meulen and Hernandez, 2013). In cortical bone, in vivo mechanical loading leads to increases in periosteal bone formation at the locations of greatest tensile and compressive strains (Torrance et al., 1994). In cancellous bone, in vivo loading has been shown to result in increases in bone density and bone volume fraction (Fritton et al., 2005; Lambers et al., 2011; Morgan et al., 2015; van der Meulen et al., 2006; Webster et al., 2010). The vast majority of studies examining in vivo mechanical loading of cancellous bone have addressed changes at the regional level (regions of cancellous bone 1–5 mm in size), yet theoretical analysis suggests that functional adaptation in bone occurs at the local level (as close as 10–100 μm) (Carter, 1982). In cortical bone, new bone formation after an acute mechanical stimulus has been correlated with local periosteal stresses and strains (Kotha et al., 2004). In cancellous bone, local mechanical stress and strain associated with long-term loading (stimulus 5 days per week for 4 or more weeks) has been shown to be moderately predictive of locations of new bone formation (conditional probability no better than 45%) (Schulte et al., 2013).

While the relationship between local tissue stress and strain and new bone formation has been established in cancellous bone

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for long-term loading (4 weeks or more of regular loading) (Schulte et al., 2013; Webster et al., 2012) it is not known how well local mechanical stimulus is associated with new bone formation over shorter time periods. Determining the spatial associations between mechanical stress/strain and new bone formation at the level of individual trabeculae immediately following an acute stimulus has the potential to provide a more direct evaluation of mechanosensory mechanisms. While stress and strain in mineralized tissue are believed to be the primary stimulus, there is evidence that fluid flow within the lacunar canalicular network (Jacobs et al., 2010) and fluid flow and pressure in the marrow space have been associated with bone formation (Hu et al., 2012; Kwon et al., 2010; Metzger et al., 2015; Qin et al., 2003; Webster et al., 2015). The ability to assess the spatial association between bone formation and mechanical stress and strain is necessary to differentiate among mechanosensory mechanisms, particularly if such measures are made soon after loading.

The long term goal of this line of investigation is to determine the mechanotransduction mechanisms in cancellous bone. Here we examine the spatial relationship between local mechanical stress/strain and bone formation in the first week after a mechanical loading stimulus. Specifically we determine the relationship between tissue stress and strain within cancellous bone and individual locations of bone formation stimulated by mechanical loading.

2. Methods

2.1. In vivo loading and micro-computed tomography

Three month old, female Sprague Dawley rats were used in this study ($n=14$). Animals were housed individually in a 12 h light 12 h dark cycle and fed standard rat chow ad libitum. Animal use was approved by the local IACUC.

Mechanical stimulus was applied to regions of cancellous bone using the rat tail loading model (Chow et al., 1993; Guo et al., 2002). The animals were anesthetized using isoflurane inhalation and threaded Kirschner wires (K-wire, dia.=1.6 mm, Zimmer, Warsaw, IN) were inserted into the seventh (Cd7) and ninth caudal vertebra (Cd9). K-wire positioning was ensured using digital radiographs (Preva, Progeny, Lincolnshire, IL). Following surgical implantation, K-wires were secured in a single piece aluminum external fixator to prevent displacement and rotation of the K-wires relative to one another, thereby unloading the eighth caudal vertebrae (Fig. 1A and B). Animals were returned to normal cage activity for 3 weeks to allow healing around the implants.

Three weeks after surgery, animals were anesthetized, K-wires were removed from the external fixator, and micro-computed tomography images (CT-120, GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) of the eighth caudal vertebrae were acquired (25 μm voxel size, 80 kVp, 32 mA, 1200 projections, 100 ms integration time). Immediately following image acquisition, the K-wires were secured into custom fixtures within a materials testing device (Bose EnduraTech LM1 test bench, Bose Corporation, Minnetonka, MN). Animals were divided into two groups, a loaded and pinned not loaded control ($n=7$ per group). Animals in the loaded group were anesthetized through isoflurane inhalation and compressive loading was applied through the two K-wires onto the eighth caudal vertebra. Loading was applied first in a ramp from 0 N to 25 N at 1 N/sec, followed by cyclic sinusoidal compressive loading from 25 N to 100 N at 0.5 Hz for 300 cycles. Preliminary assessment with strain gages indicated that the maximum applied load of 100 N was associated with surface strains of 2141 ± 644 με on the dorsal and 771 ± 534 με (mean ± SD, $n=5$) on the ventral surfaces (Goff et al., 2014). Animals in the pinned not loaded control group were anesthetized and had K-wires secured into the custom loading fixtures for the same length of time but no load was applied. The protocol was repeated once per day for 3 consecutive days and K-wires were secured in the external fixators between loading sessions. Bone formation markers were administered via intraperitoneal injection at 2 days (90 mg xylenol orange/kg body weight) and 7 days (10 mg calcein/kg body weight) after loading. Animals were euthanized 11 days post loading and bones were collected for analysis.

2.2. Finite element modeling

The distribution of tissue stress/strain within the eighth caudal vertebrae during loading was determined using high resolution finite element models generated from micro-computed tomography images (micro-CT) collected prior to loading. Following the application of a Gaussian filter (radius=1 voxel) the bone was segmented using a locally adaptive threshold based on edge detection (Waarsing et al., 2004). Finite element (FE) models were created by converting each voxel into 8 node linear brick elements using custom software (Lynch et al., 2010) (element size 25 μm which has been shown to be sufficient for modeling cancellous bone microstructure (Guldberg et al., 1998; Niebur et al., 1999)). Bone tissue was assigned a Young's modulus consistent with surface strains achieved in preliminary experiments (E=9.4 GPa) (Goff et al., 2014) and Poisson's ratio $\nu=0.3$. The intervertebral discs were included in the model as cylindrical discs attached at the ends of the bones with a diameter of 3 mm and height of 0.5 mm. Discs were modeled with $E=4.0$ MPa and $\nu=0.3$ (Elliott and Sarver, 2004). The peak load applied to the bone in vivo was simulated by applying a compressive load of 100 N across the caudal discs and constraining axial displacement at the distal disc (Fig. 1C). The finite element models were implemented with ABAQUS (Version 6, Waltham, MA) on the Gordon supercomputer (XSEDE, vSMP node, for ~140 total computing hours) (Ray et al., 2014). Models consisted of 3.85 ± 0.25 million elements (mean ± SD). Strain energy density (SED), maximum principal strain, and von Mises stress within each element was used to evaluate local mechanical stimuli. Similar trends were found with all 3 assays of local mechanical stimuli so only SED is reported for consistency with other work (Huiskes, 2000; Lambers et al., 2011; Schulte et al., 2013). Finite element models were performed for the pinned not loaded controls to provide a null control for SED distributions.

2.3. Image acquisition and processing

The eighth caudal vertebrae were collected and soft tissue was removed. The endplates were cut away with a low speed diamond saw (Isomet, Beuhler, Lake Bluff, IL) and marrow was removed using a low pressure water jet and short periods of ultrasonication. Specimens were fixed in 70% ethanol for 48 h, dehydrated in increasing concentrations of ethanol and embedded undecalcified in methyl methacrylate made opaque by the addition of sudan black dye in preparation for image acquisition with serial milling (Slyfield et al., 2012). Serial milling is a destructive imaging technique that results in a three-dimensional image of the bone and two different fluorescent markers with a voxel size of $0.7 \times 0.7 \times 5.0\ \mu m^3$ (Slyfield et al., 2009) (please see Supplementary Materials for a more detailed description of the technique). Following image reconstruction, three-dimensional

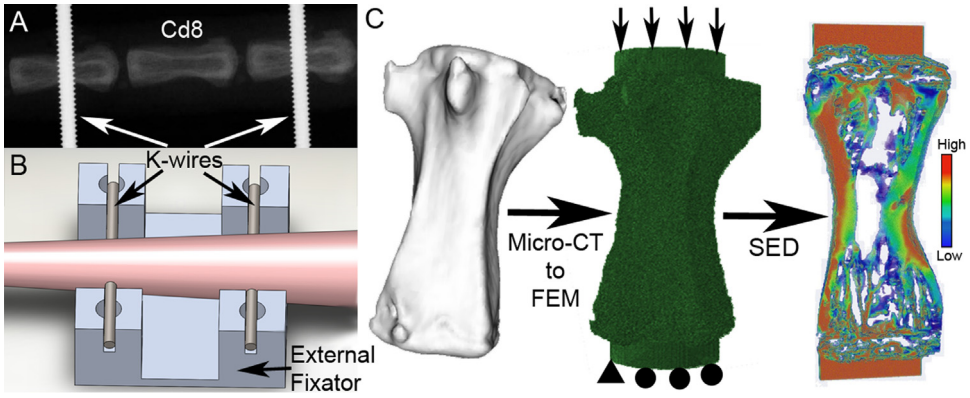

Fig. 1. (A) K-wires were inserted into the Cd7 and Cd9. (B) K-wires were secured into an external fixator between loading sessions. (C) Micro-CT images were converted to finite element models with intervertebral discs and boundary conditions as shown. The strain energy density (SED) distribution is shown for a cross section of the whole vertebra.

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