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Using digital image correlation to determine bone surface strains during loading and after adaptation of the mouse tibia

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ABSTRACT

Previous models of cortical bone adaptation, in which loading is imposed on the bone, have estimated the strains in the tissue using strain gauges, analytical beam theory, or finite element analysis. We used digital image correlation (DIC), tracing a speckle pattern on the surface of the bone during loading, to determine surface strains in a murine tibia during compressive loading through the knee joint. We examined whether these surface strains in the mouse tibia are modified following two weeks of load-induced adaptation by comparison with contralateral controls. Results indicated non-uniform strain patterns with isolated areas of high strain (0.5%), particularly on the medial side. Strain measurements were reproducible (standard deviation of the error 0.03%), similar between specimens, and in agreement with strain gauge measurements (between 0.1 and 0.2% strain). After structural adaptation, strains were more uniform across the tibial surface, particularly on the medial side where peak strains were reduced from 0.5% to 0.3%. Because DIC determines local strains over the entire surface, it will provide a better understanding of how strain stimulus influences the bone response during adaptation.

1. Introduction

The adaptive changes in bone structure that occur in response to mechanical loading are known to be a major determinant in maintaining the functional competence of bone. Numerous animal models have been developed to study both the cellular mechanisms and the architectural modifications induced by such mechanical stimuli. The turkey ulna and radius, rat ulna and tibia, and murine ulna and tibia have all been used as in vivo loading models for assessing periosteal bone adaptation (Rubin and Lanyon, 1984; Gross et al., 1997, 2002; Mosley et al., 1997; Forwood et al., 1998; Cullen et al., 2001; Robling et al., 2001; Lee et al., 2002; Mosley and Lanyon, 2002; De Souza et al., 2005). In these models, the applied load is controlled in order to deliver quantifiable mechanical sequelae. The effects of mechanical load on the tissue are normally quantified as strain at selected locations on the bone surface and are subsequently used in order to define relationships between applied loads and the architectural responses that ensue. The strain engendered in the bone is, however, often only an estimation based on measurements from one or two strain gauges attached to small, limited areas of the bone.

This is clearly a limitation in bones, which are known to exhibit inhomogeneous material properties and complex morphology and will likely not have homogeneous strain fields across the bone surface. In addition, in small bones such as the murine tibia, placement of the gauge is further constrained by the size and shape of the bone. Finite element analysis offers an alternative method for estimating the strain field, but this is also limited as it often simplifies bone material properties, geometry, and loading conditions, which can create errors in the calculated strains. Although combining strain gauge and finite element analysis provides an elegant and more robust means of estimating bone strains throughout the bone (Gross et al., 1997), there remains a need to accurately measure the mechanical strain fields generated during loading in these in vivo models. Establishing the precise characteristics of the mechanical environment, including the magnitude, gradient and distribution of strain across an entire periosteal surface, will help to establish the relationship between the mechanical sequelae and the consequent architectural modifications.

Digital image correlation (DIC) is an optical full-field technique for non-contact, 3D deformation measurements (Kahn-Jetter and Chu, 1990). In DIC, a high contrast speckle pattern is applied onto the surface of the sample and observed by the charge-coupled device (CCD) cameras during loading. The entire field of view is divided into a number of unique correlation areas, or 'facets', which typically contain a square subset of pixels. Facets track the characteristic features of the speckle pattern during loading and

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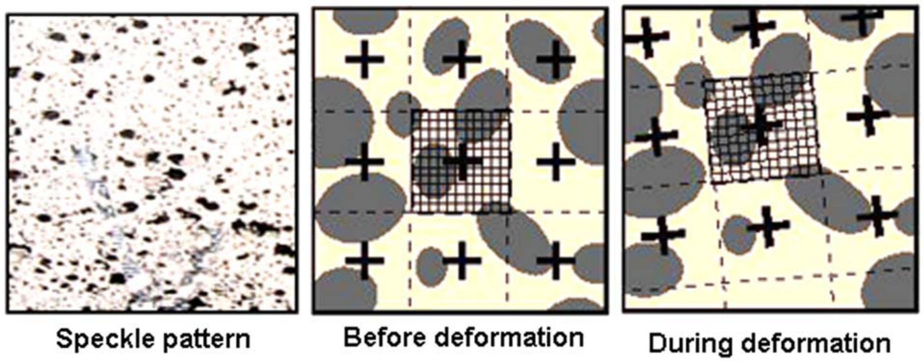

Fig. 1. Digital image correlation traces the deformation of a speckle pattern during loading. A facet, composed of a subset of image pixels represented by the grid, is used to track the speckle pattern.

provide a progressive measurement of deformation (Fig. 1). On this basis, strain averaged over a set of facets can be thought to represent a reading from a rosette strain gauge attached at that particular location to provide axial, transverse and shear strains.

DIC is particularly suitable for biological applications because it can be used for accurately determining strain in inhomogeneous, anisotropic, non-linear materials, such as bone. Nicolella et al. have previously used DIC to analyse strain distribution around a microcrack in bovine cortical bone and found that strains were largest at the crack tip and around osteocyte lacunae (Nicolella et al., 2001, 2005). Further studies have compared nanoindentation measurements of Young's modulus with local strain measured using DIC and demonstrated that both microstructure and mineral content are important determinants of local strain in cortical bone (Hoc et al., 2006). DIC has also been used to measure strain in mouse femora (Yang et al., 2007), cartilage (Zhang et al., 2005), arteries (Zhang and Arola, 2004) and bone fracture callus (Thompson et al., 2007). Despite demonstrating the utility of DIC, none of these previous studies have used samples or sections of tissue under conditions directly representative of applied in vivo loading.

In this study, we used DIC to determine strains on the surface of intact murine tibiae under compressive loading conditions, similar to those that are applied to the flexed hind limb in our experimental in vivo model. Our findings determined the sensitivity (noise) of the DIC method, generated contour maps of the strain fields on the tibial surfaces during loading, compared DIC measurements of strain with strain gauge measurements, and established the effects of load-induced adaptive changes in bone architecture on these tibial surface strains. These studies extend the utility of the murine tibial model by establishing load-induced strain fields on the surface of the bone against which architectural and cellular responses can be evaluated.

2. Methods

2.1. In vivo loading

The right tibiae of four male 8 week old C57Bl/6J mice (Charles River Company, UK) were loaded in custom built loading cups that were designed in order to allow axial compression to be applied across a flexed knee joint (De Souza et al., 2005). Briefly, the upper cup held the flexed knee and transferred the load to the tibia; the lower cup held the ankle flexed at approximately 45° (Fig. 2). Loads (12 N, cycle=0.1 s rise and fall time+10 s rest, 40 cycles/day) were applied three days a week for two weeks to anaesthetised, pre-medicated male mice. After sacrifice, both left and right hind limbs were dissected at the hip joint, soft tissues removed from the tibiae and limbs fixed in neutral buffered formalin and then preserved in ethanol. The left tibia was used as non-loaded control. Non-adapted fresh-frozen tibia (n=4) were additionally used to ensure that preservation in alcohol did not affect the strain patterns and magnitudes.

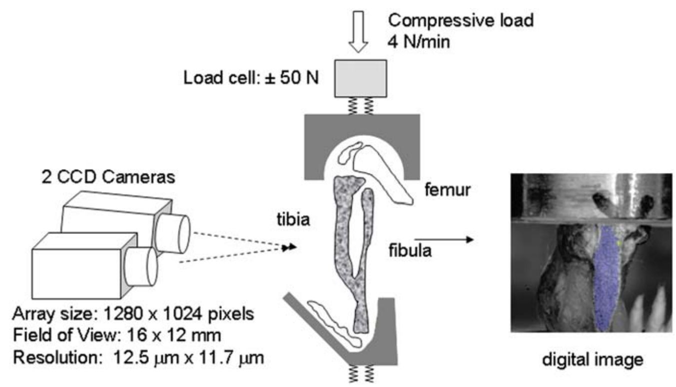

Fig. 2. Images of the mouse tibia loaded in custom made load cups were captured by 2 CCD cameras.

2.2. Digital Image Correlation

The mice limbs were rehydrated and the entire portion of the exposed periosteal surface of the tibiae coated with a thin layer of matt, acrylic water-based white paint (Deco Matt, Lefranc et Bourgeois, Le Mans, France) and then speckled with matt, water-based, black paint (Createx, Airbrush Opaque, UK), using a high-precision airbrush (Model 200, manufactured by Badger Airbrush CO, USA). The optimal size of speckles was determined from a number of initial trials. Based on the resolution of the CCD cameras (50 mm lenses with the 25 mm distance ring, Schneider Kreuznach, Bad Kreuznach, Germany), the measuring area and the intended facet size, the average pattern density was approximately 4 dots per facet, corresponding to approximately 70 dots/mm^2.

To measure strain during the application of load, the explanted limb was placed in the loading cups fastened to a universal, screw-driven materials testing machine (Instron, Model 5866, High Wycombe, UK). A $\pm$50 N load cell attached to the upper cup measured the load that was applied at a rate of 8 N/min up to a maximum axial load of 12 N. Two CCD cameras mounted on a tripod were positioned horizontally in front of the loading fixture to provide a 16 mm $\times$ 12 mm field of view (1280 $\times$ 1024 pixels, resulting in approximately 12 μm pixel size), with the depth of focus field of 6 mm. The relative position of the cameras with respect to each other was calibrated using a high-precision 10 mm $\times$ 8 mm calibration target and a pair of halogen and fluorescent lamps were installed to illuminate the specimen and enhance the visibility of its surface pattern. Prior to the actual measurement of load-induced strain on the surface of each tibia, multiple images of the non-loaded bone surface were captured in order to allow for an evaluation of the amount of experimental noise. Load-induced strains were measured twice on each side of each tibia to ensure reproducibility of the measurements. As the 2 camera system allowed viewing of only one surface, the medial and lateral surfaces of the tibiae were imaged separately. Images were collected at a frame rate of 0.5 Hz, correlated with the applied loads and an ARAMIS 1.3 M system (GOM GmbH, Germany) was used for DIC strain calculations.

Processing of the images was performed using 19 $\times$ 19 pixels square facets, with 9 pixels facet overlap, resulting in approximately 900 measurement points on each surface (lateral and medial) of the tibia and a spatial resolution of 125 μm for strain measurements. For the strain evaluation at each measurement point, the displacement values over the surrounding 5 $\times$ 5 facets were considered. Mean strain on the medial and lateral surfaces was also calculated.