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Validation of finite element models of the mouse tibia using digital volume correlation

S. Oliviero, M. Giorgi, E. Dall'Ara*

Department of Oncology and Metabolism and INSIGNEO Institute for in Silico Medicine, University of Sheffield, Pam Liversidge Building, Mappin Street, S13JD Sheffield, UK

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ABSTRACT

The mouse tibia is a common site to investigate bone adaptation. Micro-Finite Element (microFE) models based on micro-Computed Tomography (microCT) images can estimate bone mechanical properties non-invasively but their outputs need to be validated with experiments. Digital Volume Correlation (DVC) can provide experimental measurements of displacements over the whole bone volume. In this study we applied DVC to validate the local predictions of microFE models of the mouse tibia in compression.

Six mouse tibiae were stepwise compressed within a microCT system. MicroCT images were acquired in four configurations with applied compression of 0.5 N (preload), 6.5 N, 13.0 N and 19.5 N. Failure load was measured after the last scan. A global DVC algorithm was applied to the microCT images in order to obtain the displacement field over the bone volume. Homogeneous, isotropic linear hexahedral microFE models were generated from the images collected in the preload configuration with boundary conditions interpolated from the DVC displacements at the extremities of the tibia. Experimental displacements from DVC and numerical predictions were compared at corresponding locations in the middle of the bone. Stiffness and strength were also estimated from each model and compared with the experimental measurements.

The magnitude of the displacement vectors predicted by microFE models was highly correlated with experimental measurements ($R^2 > 0.82$). Higher but still reasonable errors were found for the Cartesian components. The models tended to overestimate local displacements in the longitudinal direction (R^2 = 0.69–0.90, slope of the regression line = 0.50–0.97). Errors in the prediction of structural mechanical properties were 14% ± 11% for stiffness and 9% ± 9% for strength.

In conclusion, the DVC approach has been applied to the validation of microFE models of the mouse tibia. The predictions of the models for both structural and local properties have been found reasonable for most preclinical applications.

1. Introduction

Mouse models are commonly used to investigate bone remodeling and the effect of bone treatments preclinically. In particular, the mouse tibia has been previously chosen to study the bone response to *in vivo* mechanical stimulation (Birkhold et al., 2015; Holguin et al., 2014), to ovariectomy (Klinck et al., 2008; Waarsing et al., 2004), to ageing (Buie et al., 2008; Main et al., 2010) and to pharmacological treatments, e.g. parathyroid hormone (Campbell et al., 2014; Lu et al., 2017). The gold standard for evaluating how the tibia morphology and density change over time is *in vivo* micro-Computed Tomography (microCT) imaging, which allows to acquire high resolution images of the bone at different time points and to account for intrinsic variability among animals (Bouxsein et al., 2010; Dall'Ara et al., 2016). Moreover, by converting the microCT images into micro-Finite Element (microFE) models (van Rietbergen et al., 1995) the bone mechanical behavior under loading can be predicted non-invasively. Nevertheless, before their application in preclinical assessments, such models should be validated against accurate experiments. The prediction of bone stiffness by microFE models has been extensively validated for trabecular bone specimens (Schwiedrzik et al., 2016; Wolfram et al., 2010) and human vertebral bodies (Dall'Ara et al., 2012). However, quantifying the local strains over the bone volume in a spatially resolved fashion is relevant to investigate bone adaptation. It has been shown that remodeling seems mechano-regulated by the local strains, both in the mouse tibia (Birkhold et al., 2016) and in the caudal vertebra (Schulte et al., 2013). Therefore, validating the microFE predictions at the local level is fundamental in order to obtain reliable information about the local

* Corresponding author.
E-mail addresses: s.oliviero@sheffield.ac.uk (S. Oliviero), m.giorgi@sheffield.ac.uk (M. Giorgi), e.dallara@sheffield.ac.uk (E. Dall'Ara).

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Table 1
Overview of the properties of the tested right mouse tibiae dissected from female C57BL/6J mice. For each specimen group, age, length, total bone mineral content (BMC) and tissue mineral density (TMD) are reported.

	Group	Age (weeks)	Length (mm)	BMC (mg)	TMD (mgHA/cc) Mean ± SD
Sample1	Ovariectomy	22	18.57	16.77	1078 ± 222
Sample2	Ovariectomy	22	18.70	16.66	1058 ± 216
Sample3	Wild type	24	17.95	16.74	1119 ± 228
Sample4	Wild type	24	17.09	14.26	1094 ± 221
Sample5	Wild type	16	17.76	14.51	1051 ± 225
Sample6	Wild type	16	17.63	14.23	1034 ± 219

mechanical environment engendered in the bone under loading.

Strain gauges, digital image correlation (DIC) and digital volume correlation (DVC) can be used to measure local displacements and strains of loaded bone specimens (Grassi and Isaksson, 2015). On the mouse tibia, strain gauge measurements have been performed for both determining the local strain engendered by an external load and for validating microFE predictions (Patel et al., 2014; Razi et al., 2015; Stadelmann et al., 2009; Yang et al., 2014). The main limitation of this method is that only a few strain gauges can be attached on a single tibia due to its small size (maximum of three strain gauges in (Patel et al., 2014) and in (Stadelmann et al., 2009)), and the measurement obtained represents the average strain over a relatively large area (typical size of the active gauge = 0.38 mm x 0.50 mm). Additionally, strain gauges should be ideally applied on flat surfaces, hard to find in the mouse tibia and the attachment of the sensor itself may cause a local stiffening of the specimen, as shown on the mouse forearm (Begonia et al., 2017). Digital Image Correlation (DIC) is a contactless method based on the acquisition of several images of the sample during the mechanical test, which are then used to retrieve the displacement field. The surface of the sample is conveniently speckled in order to create a random pattern, which is subsequently used to identify corresponding points in the two images based on an image correlation approach. DIC has been applied on the mouse tibia in order to quantify the distribution of strains on the surface during loading (Sztefek et al., 2010) and the sensitivity of the technique to different parameters (e.g. speckle size and density) has been analyzed (Carriero et al., 2014). DIC measurements have also been compared to microFE predictions of strains on the mouse tibia surface (Pereira et al., 2015) and on the mouse ulna and radius (Begonia et al., 2017). However, DIC can only provide measurements on a portion of the external surface of the sample. In order to overcome this limitation, Digital Volume Correlation (DVC) can be applied to two (or more) microCT images of the sample acquired during stepwise loading (Bay et al., 1999). A deformable registration approach calculates the local displacements over the whole volume of the specimen that can be differentiated into a strain field. DVC has been applied to trabecular bone samples (Chen et al., 2017; Gillard et al., 2014; Roberts et al., 2014; Zauel et al., 2005), human vertebra (Hussein et al., 2012), porcine vertebra (Costa et al., 2017), but it has never been applied on the mouse tibia. The main limitation of DVC is the need of finding a compromise between measurement accuracy and spatial resolution of the method (Dall'Ara et al., 2014, 2017). While for displacement measurements a good compromise can be found, acceptable uncertainties in strain measurements can only be obtained at very coarse resolutions (Grassi and Isaksson, 2015; Palanca et al., 2015).

The aim of this study was to use the DVC technique to validate local displacements predicted by microFE models in the mouse tibia under compression. Additionally, global stiffness and strength were estimated from microFE models and compared to the experimental measurements.

2. Materials and methods

For the validation of microFE models of the mouse tibia a similar procedure previously applied for trabecular bone (Chen et al., 2017) and porcine vertebral bodies (Costa et al., 2017) was used. Mouse tibiae were stepwise compressed within a microCT scanner, in order to acquire images of the tibiae in different loading configurations. Afterwards, a deformable registration was applied to the microCT images to compute the displacement field. MicroFE models were generated from the microCT images acquired in the preloaded configuration. Experimental and numerically predicted displacements were compared at corresponding locations. Also, global stiffness and strength predicted from microFE models were compared to the experimental measurements.

2.1. Sample preparation

Six right mouse tibiae were obtained from C57BL/6J female mice used for previous studies (Lu et al., 2015, 2017) (Table 1). Tibiae were dissected from 22-weeks-old mice which underwent ovariectomy at week 14 of age (Lu et al., 2015) (N = 2), from 24-weeks-old wild type mice (N = 2), and from 16-weeks-old wild type mice (N = 2). After carefully removing soft tissues with a scalpel, the tibiae were kept frozen at − 20 °C until testing. Total bone mineral content (BMC) and tissue mineral density (TMD) were computed from the microCT scans of the specimens as described below.

In order to align and grip the samples to the loading device, the extremities of the tibiae were embedded in resin (Technovit 4071, Kulzer, Germany) (Fig. 1A). Dissected tibiae were defrosted at room temperature in saline solution for 2 h and subsequently dehydrated in air for 1 h for the embedding. The total length was measured using a caliper. The longitudinal axis of the tibia was visually aligned to a vertical reference and the distal end was embedded in resin until the 10% of the total length. The same procedure was applied to embed the proximal end. After embedding both ends in resin, the tibia was frozen again until testing.

2.2. Stepwise compression tests and microCT imaging

Each embedded tibia was defrosted, rehydrated in saline solution for 3 h and then placed in the loading device (Fig. 1B) wrapped in a saline solution-soaked gauze, in order to avoid dehydration during the test. The loading device was placed into a microCT system (VivaCT 80, Scanco Medical, Bruettisellen, Switzerland). Stepwise compression tests were performed by means of a screw-ball joint and the axial load was measured with a 100 N load cell (C9C, HBM, United Kingdom). In order to reduce the effect of relaxation, at each load step the microCT image acquisition was started after 25 min. The procedure was repeated for four different load levels (Fig. 1C): axial load of 0.5 N to avoid moving artifacts during the scan (hereafter referred to "Preload"); axial load of 6.5 N in the elastic range, defined as half of the typical one applied during *in vivo* loading of the mouse tibia (De Souza et al., 2005) (hereafter referred to "LoadStep1" or "LS1"); axial load of 13.0 N, representative of a typical load applied in *in vivo* tibia loading experiments (De Souza et al., 2005) (hereafter referred to "LoadStep2" or "LS2"); axial load of 19.5 N to study the inelastic range (hereafter referred to "LoadStep3" or "LS3"). In total four microCT images were acquired for each sample. After the stepwise compression test, the tibia