Journal of Biomechanics 43 (2010) 557–562

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

A finite element inverse analysis to assess functional improvement during the fracture healing process

Jared A. Weis [a,b], Michael I. Miga [b], Froilán Granero-Moltó [a], Anna Spagnoli [a,c,*]

[a] Department of Pediatrics. University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
[b] Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37232, USA
[c] Department of Biomedical Engineering University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Accepted 2 September 2009

Keywords:
Fracture healing
Finite element analysis
Material properties
Biomechanical testing
Micro-CT

ABSTRACT

Assessment of the restoration of load-bearing function is the central goal in the study of fracture healing process. During the fracture healing, two critical aspects affect its analysis: (1) material properties of the callus components, and (2) the spatio-temporal architecture of the callus with respect to cartilage and new bone formation. In this study, an inverse problem methodology is used which takes into account both features and yields material property estimates that can analyze the healing changes. Six stabilized fractured mouse tibias are obtained at two time points during the most active phase of the healing process, respectively 10 days (n=3), and 14 days (n=3) after fracture. Under the same displacement conditions, the inverse procedure estimations of the callus material properties are generated and compared to other fracture healing metrics. The FEA estimated property is the only metric shown to be statistically significant (p=0.0194) in detecting the changes in the stiffness that occur during the healing time points. In addition, simulation studies regarding sensitivity to initial guess and noise are presented; as well as the influence of callus architecture on the FEA estimated material property metric. The finite element model inverse analysis developed can be used to determine the effects of genetics or therapeutic manipulations on fracture healing in rodents.

1. Introduction

Approximately 10–20% of the 6.2 million annual bone fractures result in non-unions, causing significant morbidity and mortality (Einhorn, 1995; Marsh, 1998). In long-bones, fracture healing proceeds through the formation of a cartilaginous template that is then replaced by bone that undergoes remodeling (Einhorn, 1998). A critically important function of bone healing is that the healing tissue provides sufficient mechanical stabilization such that a return to functionality is possible. Experimental studies on fracture healing have largely been dependent on rodent models. However, the lack of sensitive methods to monitor and relate the fracture mechanical properties with tissue type renders those studies inadequate to fully evaluate the fracture healing patho-physiology.

Assessment of fracture healing has relied on histological, imaging, and biomechanical testing (BMT) (Gerstenfeld et al., 2005). Histological methods allow the visualization of tissue-specific molecules over histological sections by in-situ hybridization, immunohistochemistry, or specific staining. However, comparisons between sections are difficult and true quantitative assessment is unrealistic. Furthermore, histological methods are limited to *post-mortem* analysis and cannot provide functional information. Various imaging modalities have been used to assess the fracture healing, such as micro-computed tomography (μCT), magnetic resonance, and positron emission tomography (Cattermole et al., 1996; Grigoryan et al., 2003; Ciprian et al., 2004; Lynch et al., 2004; Severns et al., 2004; Schmidhammer et al., 2006; Hsu et al., 2007; Saran and Hamdy, 2008). μCT imaging is mostly used due to advantages in 3D reconstructions. However, imaging provides no information about tissue types and mechanical properties. BMT remains the gold standard for the functional assessment of fracture healing. Standard BMT analyses use force versus displacement data and analytic calculations based on beam theory to generate mechanical property information. Beam theory calculations rely on the assumption of a homogeneous cross section, but because of the irregular geometry of the callus, these calculations are strongly biased by geometrical factors (van Lenthe et al., 2008).

Some studies have explored coupling μCT imaging with finite element analysis (FEA) to predict the mechanical behavior based on geometrical information. In particular, studies have evaluated

* Corresponding author at: Department of Pediatrics, Division of Pediatric Endocrinology, 3341 Mason Farm Road, Campus Box: 7039, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599-7039, USA.. Tel.: +1 919 843 6904; fax: +1 919 843 6905.
E-mail address: spagnoa@med.unc.edu (A. Spagnoli).

0021-9290/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbiomech.2009.09.051

μCT attenuation to stiffness value transformations to provide material properties and found empirical power law relationships between modulus and bone mineral content assessed by μCT attenuation/density (Bourne and van der Meulen, 2004; Shefelbine et al., 2005). Shefelbine and colleagues have also reported a weak correlation between predicted and experimental torsional rigidity with a very poor predictive value in calluses studied at early healing stages when mineralization is low (Bourne and van der Meulen, 2004; Shefelbine et al., 2005). It is quite apparent that the direct relationship between μCT attenuation/density and mechanical parameters is unclear and is to some degree unsatisfactory; and when factoring in the potential for variability of this relationship across experimental systems, it is unlikely that the correlation will improve.

In our studies, rather than using a μCT-to-stiffness empirical relationship, we have used an elastographic approach to directly generate values for mechanical parameters. Our approach combines an inverse finite element model of the subject's cartilage/bone geometry (μCT/histological imaging data), data acquired from BMT, and numerical optimization techniques to characterize the callus mechanical properties. This approach does not require calibration per system but rather is an active reconstruction parameter that can be measured experimentally. The concept of an 'inverse' FE analysis method to determine the mechanical parameters to monitor the progression of fibrogenic diseases has been demonstrated. These techniques are more widely referred to as elastography (Ophir et al., 1991; Greenleaf et al., 2003; Washington and Miga, 2004; Miga et al., 2005; Barnes et al., 2007; Samani and Plewes, 2007; Ou et al., 2008). Within this work, the approach is used to evaluate mechanical properties as a biomarker in the assessment of fracture healing progression. Quantifying the change in mechanical properties during the fracture healing process may provide information that: (1) allows to determine when healing has failed to progress, (2) suggests the need for intervention in non-union/slow healing fractures, and (3) evaluates the effectiveness of treatments that aim to enhance the healing process through the formation of more mechanically competent tissue.

2. Methods

2.1. Generation of the computational model

An inverse FEA procedure was developed to determine the stiffness of the callus based on μCT imaging and BMT data. As summarized in Fig. 1, the procedure begins with the establishment of an assumed Hookean linear elastic tissue model framework for the bone/callus system. The process continues with the development of a bone/callus computer model of the subject generated from μCT image volumes. A volumetric tetrahedral grid is then generated to represent a FE mesh system.

The boundary conditions for the model were chosen to reflect the BMT protocol, in which the top boundary is prescribed a fixed upward normal displacement with no lateral displacement (Dirichlet boundary conditions). The bottom surface was also fixed in both the normal and lateral direction. The remaining boundary conditions for the sides of the model were stress free. The displacement criteria selected for each sample was based on the individual force/displacement curve obtained from BMT. A series of four displacements were taken along the curve at 25%, 50%, 75%, and 100% of the linear elastic limit to reproduce the linear portion of the curve. As pointed out in Fig. 5, the linear elastic limit was defined as the point at which the curve exhibited plastic deformation (slope ≤ 0 in our case). Solutions to the elastic system are then generated as reported previously (Barnes et al., 2007). As shown by Barnes and colleagues, the unused Galerkin equations associated with the implementation of the Dirichlet boundary conditions are utilized post model-execution to estimate the local boundary stress (Barnes et al., 2007). This stress is then averaged over the tensile boundary surface and multiplied by the surface area to generate a model-calculated average force (F_{calc}) applied to the bone surface for the given displacement. The model is solved at each displacement value to generate 4 model-calculated average forces which are compared to the corresponding forces measured from the force/displacement curve in a least squares sense and properties of the callus determined through an iterative optimization process. A further discussion of the inverse problem framework is discussed the Supplemental material.

2.2. Experimental model

2.2.1. Mouse stabilized tibia fracture model

Female FVB-NJ mice (Jackson Laboratories) 8–12 weeks old were anesthetized using isofluorane to provide deep anesthesia. Pin-stabilized mid-diaphyseal tibia fractures were generated by insertion of a 0.25 mm stainless steel pin (Fine-Science-Tools) through the tibial tuberosity followed by fracture creation using a three-point bending device with a standardized force. Immediately following tibia fracture, 0.5 mg/kg of bupremorphine was administered for pain control. On post-fracture days 10 and 14, mice were euthanized, fractured tibias were dissected and wrapped in PBS soaked gauze and stored at −80 °C until further analysis. Animal studies were approved by the Institutional Animal Care and Use Committee at Vanderbilt University Medical Center and the University of North Carolina at Chapel Hill.

2.2.2. μCt Callus Imaging and μct/Histological Thresholding analyses

μCT scans were performed using a Scanco μCT 40 scanner (Scanco Medical) and were obtained at 55 kVp, 145 μA, 300 ms integration time using 12 μm voxel resolution along 5.2 mm length centered at the fracture line (Reynolds et al., 2007). μCT reconstructions were used for subsequent FEA and volume measurements. To determine material type (newly mineralized bone, highly mineralized bone and cartilage) and quantify callus volumes from μCT scans, a parametric thresholding study was performed by serial μCT scanning and histological analysis as more extensively reported within the Supplemental Material.

2.2.3. BMT analyses

Fractured tibia ends were embedded into a polymethylmethacrylate cast using custom designed testing fixtures, leaving the fracture callus exposed. Specimens were kept fully hydrated with PBS during the entire testing procedure. The fixtures were loaded into an Enduratec Electroforce 3100 mechanical tester (Bose, Enduratec Systems Group) and tested in tension at a fixed displacement rate of 0.25 mm/min using a 22 N transducer (Honeywell Sensotec) for force data (Colnot et al., 2003). Displacement and force were recorded until failure and used for subsequent FEA and to determine biomechanical metrics of fracture healing. Additional descriptions can be found in the Supplementary Material.

2.2.4. Generation of subject-specific FE models

Subject-specific FE models were generated for 6 tibias (three each at 10 and 14 days post-fracture). After using the imaging protocol above, μCT image sets were semi-automatically segmented and boundary descriptions (as described by 3D points and 3D triangular patches) were generated through the use of a marching cubes algorithm in a commercially available image analysis software (Analyze, AnalyzeDirect) for both the entire bone/callus and solely the cortical bone. Boundary descriptions of each were then used to create a heterogeneous FE tetrahedral mesh consisting of two properties (i.e. cortical bone and other material) using custom-built mesh generation methods (Sullivan et al., 1997). Once the 3D mesh is created, an image-to-grid approach is utilized which determines the voxel intensities within each tetrahedral element from the imaging domain and assigns properties based on thresholding.

Values of Poisson's ratio were assumed for all tissue types (0.3 for bone and 0.45 for callus) based on the literature (Shefelbine et al., 2005) and values associated with the near-incompressible nature of soft tissue. In addition, the cartilage and low-mineralized bone were lumped into a single isotropic property. The value of the void space elastic modulus was assumed as 0.1 Pa (many orders of magnitude below callus value). Reported values of the cortical bone modulus range from ~4 GPa to ~21 GPa (Choi et al., 1990; Jamsa et al., 1998; Schriefer et al., 2005). Because of this large variability, we tested the inverse FEA modulus estimations to explore the impact of different cortical bone modulus values using respectively 5, 10 and 15 GPa. As reported in Supplemental Table 1, we found that the estimated callus elastic modulus did not change with the assumed cortical bone modulus (maximum of ~4.5% difference, not statistically significant). Thereafter, the 5 GPa value has been used in all the studies performed.

2.3. Simulation studies

A cylinder mesh with three layers was created to simulate a simplified appearance of a bone fracture callus, as seen in Fig. 2. The simplified geometry allows analytic comparisons to FEA results. Simulations were then performed on the cylinder mesh to test the accuracy and sensitivity of the inverse FEA procedure upon initial guess, with material properties approximating that of bone and callus (5 GPa and 1 MPa, respectively) and radius and total height of 1 and 6 mm, respectively. To gauge accuracy of the simulations, the forward elastic model was used to calculate boundary normal surface forces for a step displacement corresponding to 0.5% strain and compared to an analytic calculation of the