

Exploring strain amplification in osteocytes: a combined confocal microscopy and finite element approach

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INTRODUCTION

Bone is an adaptive material, which is particularly responsive to mechanical loading and can adapt its mass and structure to meet the mechanical demands experienced throughout life. Osteocytes are believed to act as strain sensors that regulate bone adaptation in response to changes in the mechanical environment. Theoretical models have sought to predict the mechanical stimuli experienced by osteocytes under load-induced fluid flow in vivo [1]. Recent computational models of osteocytes in 3D corroborate these analytical predictions [2, 3]. It has been proposed that the pericellular matrix and ECM projections might amplify strain at the cellular membrane to levels known to initiate an osteogenic response [4]. However these studies employed idealised geometries, which do not accurately represent osteocyte geometry in vivo [5]. Therefore the precise mechanical stimuli that bone cells experience in vivo are not fully understood and strain amplification theories are not validated.

Validation of computational models of individual osteocytes is challenging due to the fact that osteocytes are embedded in a mineralized matrix. Methods developed by Nicoletta et al [6, 7] have provided high resolution imaging of bone under mechanical loading using a custom microscopy loading frame with inverted fluorescence microscopy. However these studies were limited to 2D imaging of cells on an exposed optical microscopy plane. A confocal microscopy indentation system for studying in situ chondrocyte mechanics has been developed [9] but, although methods have been developed for micro-mechanical testing of microscopic bone tissue specimens [8], no such device exists for studying in situ osteocyte mechanics.

The objective of this research is to use computational methods to predict the physiological loading conditions experienced by bone cells during normal physiological activities. We employ confocal microscopy techniques to develop geometrically accurate 3D finite element models of osteocytes in vivo. Additionally we design a customised loading rig for confocal microscopy to image osteocytes in 3D under physiological loading conditions. As well as providing validation of our computational methods, this experimental technique provides a unique insight into the mechanical environment of osteocytes in situ.

MATERIALS AND METHODS

Computational Model: Transverse sections of rat tibia were fixed in formalin and stained with fluorescein isothiocyanate (FITC) solution to visualise the peri-cellular space [10]. Confocal scans (Zeiss LSM 51 confocal microscope) were taken with a 40x oil immersion lens and wavelength excitation of 488 nm. Fluorescent osteocytes are imaged by minimising exposure times and using a rapid scan speed through the depth of the section, see Figure 1a. Four finite element models of individual osteocytes and their surrounding extracellular and pericellular matrices were generated from confocal images using MIMICS voxel-meshing software. The osteocyte cell membrane ($E=4.47\text{kPa}$, $\nu=0.3$), the surrounding pericellular matrix ($E=40\text{kPa}$, $\nu=0.4$) and the extracellular matrix (ECM) ($E=16\text{GPa}$, $\nu=0.37$) were modeled as linear elastic materials using ABAQUS finite element software. Physiological compression levels of 500, 1500, and 3000 $\mu\epsilon$ were applied to the ECM surrounding the cell. An idealised geometry of an osteocyte was also generated for comparison. ECM projections into osteocyte canaliculi were included to investigate their strain amplification role.

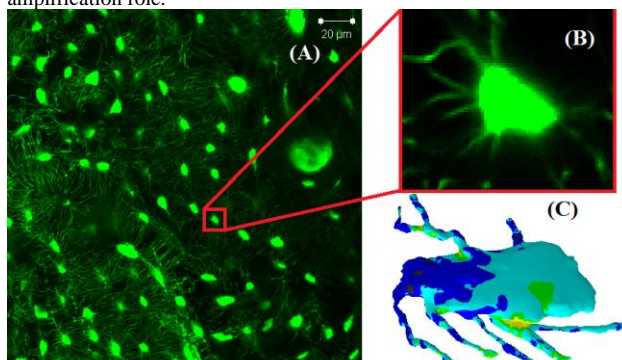


Figure 1: (A) Confocal microscopy scan of the lacunar-canalicular network with (B) an individual osteocyte, (C) a finite element contour plot of the osteocyte (under 3000 $\mu\epsilon$)

Experimental Validation: To validate our computational models we carry out experimental analyses of microscopic bone specimens. Long bone sections (10 mm length, 0.5 mm thickness) are cut from the medial section and stained with FITC. These are placed in a custom loading device positioned under the objective of a confocal microscope, see Figure 2. Mechanical loading is applied to bone sections in successive load steps (corresponding to strains of 500, 1500, and 3000 $\mu\epsilon$) and confocal imaging is carried out to simultaneously image osteocytic lacunae below the periosteum, see Figure 1a. FE model predictions are compared to experimental results to validate the assumptions.

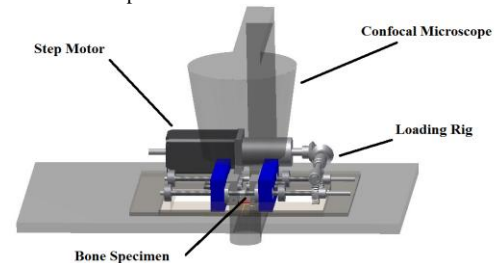


Figure 2: Custom-built loading rig under a confocal microscope

RESULTS

The results in Figure 3 show that the four realistic osteocytes experience at least 30% greater strain throughout their volume than the idealised models. Qualitatively, higher strains are observed in cell processes compared to the cell body (see Figure 1c). The inclusion of a pericellular matrix results in 9-15% greater strain transfer into the osteocyte. The presence of bony projections has been shown to impart approximately 50% more strain stimulus to the osteocyte, highlighted in Figure 1c, suggesting a strain amplification role.

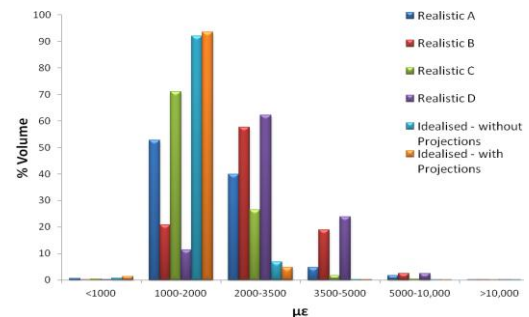


Figure 3: Strain distribution within osteocytes (under 3000 $\mu\epsilon$)

DISCUSSION

This study uses a computational approach to investigate strain amplification at the level of a single osteocyte. Cell culture studies have shown that osteocytes require strains at approximately 10,000 $\mu\epsilon$ to elicit a biochemical response. Using physiologically realistic models of osteocytes our results provide direct evidence that (1) cell processes experience greater mechanical stimulation than the cell body, (2) the pericellular matrix amplifies strain to the cell body by 9-15% and (3) ECM projections amplify strain to the cell body by approximately 50%. Interestingly strains in excess of 10,000 $\mu\epsilon$ are observed along cell processes which are significantly greater than surface strains of 2000-3000 $\mu\epsilon$ reported for whole bones [11]. Most interestingly the realistic geometries and the pericellular matrix were both shown to generate these high strain levels throughout a greater proportion of the cell, suggesting that anatomically accurate models provide a clearer understanding of the in situ mechanical environment of the osteocyte. Our findings provide direct evidence that PCM and ECM constituents play important roles in bone mechanobiology [5].

Ongoing experimental validation of these computational models will significantly advance understanding of bone mechanobiology by enabling prediction of the physiological loading conditions experienced by osteocytes in vivo.

REFERENCES: [1] Han et al., PNAS, 2004,101 [2] McCreddie et al., J Biomech, 2004 37 [3] Bonivitch et al., J Biomech, 2007, 40 [4] Anderson et al., J Biomech, 2008, 41 [5] Wang et al., PNAS, 2007 104(40) [6] Nicoletta et al., J Biomech, 2006, 39 [7] Nicoletta et al., J Biomech, 2001, 34 [8] McNamara et al., J Biomech, 2006, 39 [9] Nicoletta et al., Eur J Morphol, 2005, 42 [10] Ciani et al., Bone, 2009, 44 [11] You et al., J Biomech., 2001, 34

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