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Fluid Flow in the Osteocyte Mechanical Environment:

A Fluid-Structure Interaction Approach

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ABSTRACT

Osteocytes are believed to be the primary sensor of mechanical stimuli in bone, which orchestrate osteoblasts and osteoclasts to adapt bone structure and composition to meet physiological loading demands. Experimental studies to quantify the mechanical environment surrounding bone cells are challenging and as such computational and theoretical approaches have modelled either the solid or fluid environment of osteocytes to predict how these cells are stimulated in vivo. Osteocytes are an elastic cellular structure that deforms in response to the external fluid flow imposed by mechanical loading. This represents a most challenging multi-physics problem in which fluid and solid domains interact and as such no previous study has accounted for this complex behaviour. The objective of this study is to employ fluid-structure interaction (FSI) modelling to investigate the complex mechanical environment of osteocytes in vivo. Fluorescent staining of osteocytes was performed in order to visualize their native environment and develop geometrically accurate models of the osteocyte in vivo. By simulating loading levels representative of vigorous physiological activity (3,000 με compression and 300 Pa pressure gradient), we predict average interstitial fluid velocities (~60.5 μm/s) and average maximum shear stresses (~11 Pa) surrounding osteocytes in vivo. Interestingly these values occur in the canaliculi around the osteocyte cell processes and are within the range of stimuli known to stimulate osteogenic responses by osteoblastic cells in vitro. Significantly our results suggest that the greatest mechanical stimulation of the osteocyte occurs in the cell processes, which cell culture studies have indicated is the most mechanosensitive area of the cell. These are the first computational FSI models to simulate the complex multi-physics mechanical environment of osteocyte in vivo and provide a deeper understanding of bone mechanobiology.

1. INTRODUCTION

Bone is a highly efficient material, capable of remodelling its mass and structure in response to everyday mechanical loading. This adaptive behaviour is believed to be orchestrated by osteocyte cells, which recruit osteoblasts and osteoclasts to adapt bone structure and composition in response to changes in physiological loading. In vitro cell culture studies have suggested that osteoblasts and osteocytes act as sensors of mechanical stimuli in bone (Owan et al. 1997; Smalt et al. 1997), but that osteocytes are the most sensitive bone cell type to mechanical stimulation (Ajubi et al. 1996; Klein-Nulend et al. 1995; Westbroek et al. 2000), and also the most influential for stimulating osteogenesis (Birmingham et al. 2012b).

The precise mechanical stimuli that osteocytes experience in vivo has been much debated. Experimental studies point to loading-induced fluid flow around the osteocyte as the primary stimulus for bone growth (Owan et al. 1997; Smalt et al. 1997; You et al. 2000). Theoretical studies have predicted that loading the bone matrix surrounding osteocytes generates a pressure differential that drives flow of interstitial fluid within the lacunar-canalicular network (Weinbaum et al. 1994; Han et al. 2004; Wang et al. 2005; Wang et al. 2007; You et al. 2001). The fluid flow generates a shear stress on the osteocyte cell membrane and this is thought to act as a stimulus for biochemical signalling (Knothe Tate and Knothe 2000; Knothe Tate et al. 1998a; Knothe Tate et al. 1998b; Knothe Tate et al. 2000; Wang et al. 2000), a theory substantiated by experimental observations of bone cells responding to flow derived shear stress and not streaming potentials or chemotransport (Bakker et al. 2001). In vitro cell culture studies, in which osteoblastic cells were exposed to various mechanical stimuli, have suggested that load-induced interstitial fluid flow may stimulate a stronger osteogenic response than direct mechanical strain in bone cells (Owan et al. 1997; Smalt et al. 1997; You et al. 2000). Recent in vitro studies have observed osteogenic responses in

osteocyte-like cells exposed to fluid shear stress levels between 0.4 and 1.2 Pa within in vitro flow experiments (Bacabac et al. 2004; Bakker et al. 2001), while previous studies have similarly shown a bone growth response in cells exposed to substrate strains greater than 10,000 με (Burger and Veldhuijzen 1993; You et al. 2000). Furthermore mechanical stimulation has been shown to enhance bone tissue regeneration in vitro to a certain extent (Sittichockechaiwut et al. 2009). However, existing tissue regeneration approaches do not strive to mimic the in vivo mechanical environment surrounding cells, primarily because these stimuli are unknown. It is likely that mechanobiology based approaches for bone tissue regeneration would be enhanced if levels of mechanical stimulation applied to cells in vitro were analogous to those experienced in vivo during bone growth. Therefore characterisation of the mechanical stimulus experienced by osteocytes within the lacunar-canalicular network is necessary.

As osteocytes are embedded in a mineralised extracellular matrix, direct experimental studies are challenging. Researchers have sought to overcome this problem by developing mathematical and analytical models of interstitial fluid flow in bone. Initially bone was treated as a biphasic continuum, with the application of Biot's poroelastic theory (Biot 1941; Biot 1955), and these studies predicted that pressure gradients resulting from mechanical loading generated fluid flow around the osteocyte (Piekarski and Munro 1977). Analytical models of idealised osteocyte canaliculi under load-induced fluid flow have been developed and applied to predict the in vivo range for shear stress (0.8-3 Pa) and deformation of osteocyte cell membranes (Han et al. 2004; Weinbaum et al. 1994; You et al. 2001; Zeng et al. 1994). Recent analytical models have suggested that interstitial fluid viscosity and pericellular matrix permeability are the dominant parameters affecting flow in the lacunar-canalicular network (Sansalone et al. 2012). Computational finite element modelling techniques have also been applied to idealised models of the lacunar-canalicular system and

have predicted abrupt changes in the drag forces within the canaliculi arising from changes in geometry or proximity to bone microporosity and the Haversian canals (Mak et al. 1997). One study investigated the fluid environment of an idealised osteocyte and predicted high shear stresses within the canaliculi whereas the osteocyte cell body is primarily exposed to hydrodynamic pressure (Anderson et al. 2005). Recent studies identified projections of the extracellular matrix into the pericellular space around the canaliculi (McNamara et al. 2009), and theoretical and computational studies have suggested that such projections amplify the strain stimulus to the osteocyte (Han et al. 2004; Verbruggen et al. 2012; Anderson and Knothe Tate 2008). Ultra high voltage electron microscopes (UHVEM) have been used to develop highly detailed computational models of 80 nm long sections of osteocyte canaliculi of canaliculi, and these have been applied to predict that the geometry of the pericellular space greatly affects the velocity of fluid flow around the osteocyte cell processes (Kamioka et al. 2012).

In all previous computational models bone cells and tissue were modelled using either solid mechanics approaches (where extracellular fluids were modelled using poroelastic assumptions) or fluid dynamics modelling, wherein the biological tissues were assumed to be rigid for the purposes of understanding fluid flow and shear stresses. In reality, bone cells are composed of an elastic cell membrane that deforms in response to the external fluid flow imposed by mechanical loading (Knothe Tate 2003). The assumption that osteocytes are static rigid bodies precludes the elucidation of cellular strains arising from fluid flow in the lacunar canalicular network (Anderson et al. 2005; Anderson and Knothe Tate 2008; Kamioka et al. 2012). To fully simulate this behaviour represents a most challenging multi-physics problem, which is too complex to solve analytically and requires a fluid-structure interaction (FSI) approach.

Furthermore previous studies have used idealised geometries (Anderson et al. 2005; Rath Bonivtch et al. 2007) or have modelled limited portions of the cell (Anderson and Knothe Tate 2008), but computational models have recently shown that the use of idealised geometries predicts stress and strain values which are significantly lower than those predicted by geometries developed from physiological imaging (Anderson and Knothe Tate 2008; Verbruggen et al. 2012). In vitro experimental studies have shown that the geometry of the bone cell affects its response to strain (Bacabac et al. 2008). Therefore an approach which examines the effect of the complex architecture of the lacunar-canalicular network on fluid shear stress and cellular strains in osteocytes is required.

The objective of this research is to use computational methods to predict the mechanical environment of osteocytes in vivo under physiological loading. Using representative models of the osteocyte and its environment with fluid structure interaction (FSI) techniques we examine both shear stresses on the cell membrane and resulting strain within the osteocyte arising from mechanically-driven interstitial fluid flow in vivo.

2. MATERIALS AND METHODS

2.1. Model generation

Fluorescent staining of thick transverse sections of the mid-diaphysis of the tibia of a male 6-8 month old Sprague-Dawley rat was performed, using FITC (Fluorescein Isothiocyanate isomer I, Sigma-Aldrich) solution at 1 per cent in ethanol to visualize the pericellular space (Verbruggen et al. 2012). Confocal scanning of osteocytes was performed using a confocal microscope (Zeiss LSM 51) with a 40x oil immersion lens at laser wavelength excitation of 488 nm, allowing visualisation of their native environment. Confocal microscopy allowed acquisition of a z-stack of scans through the depth of the sample, taken at 2048 x 2048 x 47 pixels with a field of view of 255 µm, resulting in a resolution of 0.1 µm in x-y plane and 0.6 µm in the z axis, that were then used to develop geometrically accurate models of osteocytes as previously described (Verbruggen et al. 2012). Briefly, MIMICS (Materialise) image processing software was used to generate 3D reconstructions of the images, with thresholding to between -884 and -769 Hounsfield units to allow segmentation of the lacunar-canalicular space form the surrounding matrix (Verbruggen et al. 2012). These models were then meshed using 3-Matic (Materialise) voxel-meshing software to create four solid osteocyte models with anatomically accurate geometries (Verbruggen et al. 2012). Boolean operations were applied to generate a surrounding extracellular matrix (ECM) and pericellular fluid space (PCS), offset from the osteocyte model by 0.08 µm, based on experimental measurements of the geometry of the osteocyte environment (McNamara et al. 2009; Wang et al. 2005; You et al. 2004). These models were meshed with ANSYS SOLID72 tetrahedral elements and exported to ANSYS. Models of an idealised osteocyte and an idealised osteocyte with ECM projections were generated to compare with the representative geometries (Verbruggen et al. 2012), similar to

the methods described above. The four representative geometries and the idealised geometry are shown in Fig. 1 (A-D) and Fig. 1 (E) respectively.

2.2. Fluid-structure interaction analyses:

2.2.1. Solid Material and Fluid Properties

All solid structures were modelled as linearly elastic, isotropic materials. An elastic modulus of 16 GPa and Poisson's ratio of 0.38 was attributed to the ECM (Deligianni and Apostolopoulos 2008). A modulus of 4.47 kPa and Poisson's ratio of 0.3 was attributed to the osteocyte cell body and processes (Sugawara et al. 2008).

The properties of the interstitial fluid were assumed to be similar to salt water, with a density of 997 kgm^{-3} and a dynamic viscosity of 0.000855 $kgm^{-1}s^{-1}$ (Anderson et al. 2005). Flow within the lacunar-canalicular system was assumed to be laminar in nature.

2.2.2. Boundary conditions and loading

Two-way fluid-structure simulations were conducted using coupled ANSYS Multi-physics software to investigate the behaviour of interstitial fluid under physiological conditions. Two levels of fluid-solid interactions are performed to simulate interaction between the ECM and the interstitial fluid and also between this fluid and the osteocyte cell membrane.

The initial two-way FSI simulation is conducted through a bi-directional coupling of the ANSYS CFX solver to the ANSYS Structural finite element (FE) solver. A displacement boundary condition was applied to model faces to generate a compressive load of 3,000 με, representative of vigorous physiological loading (Burr et al. 1996). Due to the fact that mechanical loading in bone occurs on the whole organ level, with compression and tension occurring in different regions driving fluid across the bone network, a pressure gradient is

applied across the models to represent this (Knothe Tate 2003; Manfredini et al. 1999; Steck et al. 2003; Knothe Tate and Niederer 1998). Therefore an inlet pressure of 300 Pa was assigned to the inlets on one face and the remaining inlets were defined as outlets at a relative pressure of 0 Pa (Manfredini et al. 1999; Steck et al. 2003; Knothe Tate and Niederer 1998), similar to the pressure gradient applied by Anderson et al. (Anderson et al. 2005).

While in vivo loading is of bone is dynamic and cyclic (Fritton et al. 2000), the linear elastic material properties used here allowed simplification of loading to ramped static loading. Loading was applied uni-axially and symmetrically, to represent longitudinal compression of long bones in vivo (Taylor et al. 1996), with opposing faces constrained symmetrically to prevent rigid body motion. Using a staggered iteration approach inherent in ANSYS coupling software, the deformations at the interface between the ECM and the PCS resulting from the applied loading are mapped onto the fluid domain. The resulting fluid equations are solved and forces are relayed back to the solid ECM domain as new boundary conditions, allowing gradual mesh motion and strongly coupled solution through further iterations. This staggered iteration approach is performed repeatedly within each step until convergence of the field equations and a fully implicit solution is achieved. Upon solution of this FSI analysis, the loading-induced fluid flow can be analysed. The pressure load on the cell membrane arising from the flow is then exported to ANSYS Structural and interpolated onto the surface of the solid osteocyte domain. This FSI analysis facilitates investigation of the deformation and strain in the cell generated by the interstitial fluid boundary conditions imposed by global matrix loading.

3. RESULTS

The complex multi-physics nature of this study allows analysis of multiple aspects of the osteocyte mechanical environment, which are examined in sequence here.

3.1. Interstitial fluid velocity:

The streamline plots in Fig. 2B and Fig. 3 show the velocity distribution on the surface of representative osteocytes. Qualitatively it can be seen from these images that regions of highest velocities are located within osteocyte canaliculi, with maximum velocities of 238.1-325.7 μm/s (σ = 298.48 ± 35.84 μm/s) in representative models and 219.2 μm/s in the idealised model. In contrast fluid velocities were much lower surrounding the osteocyte cell body, with magnitudes of approximately 38-79 μm/s (σ = 60.58 ± 16.5 μm/s) predicted in the representative models and approximately 27 μm/s observed in the idealised models (see Fig. 7A). It can also be seen that the fluid velocity within canaliculi is affected by variations in surface roughness of the canalicular wall. This is noticeable in the effect of ECM projections on fluid flow in the osteocyte canaliculi as shown in Fig. 6. Fluid velocity is seen to increase in these areas in both the representative and idealised osteocytes (Fig. 6A and 6C).

3.2. Shear stress on the cell membrane:

The shear stress distribution on the surface of the representative osteocytes resulting from the interstitial fluid flow is shown in Fig. 2C and Fig. 4. Visually the highest shear stresses are predicted within the osteocyte canaliculi, resulting in stress concentrations on the cell processes (see Fig. 6B). A similar effect is seen in idealised models when ECM projections are included (see Fig. 6D). Maximum shear stresses are shown in Fig. 7B, and are observed to increase to 6.9 Pa in the idealised models with the inclusion of ECM projections in the osteocyte canaliculi. The representative models experience even higher levels of shear stress stimulus, with maximum shear stresses of approximately 9.5-15.2 Pa (σ = 11.6 ± 2.5 Pa) located in the canaliculi. All osteocyte models experienced shear stress above the level of

shear stress required for bone growth (0.8 Pa) (Han et al. 2004; Weinbaum et al. 1994; You et al. 2001; Zeng et al. 1994), with the amount of the cell membrane stimulated to this level shown in Fig. 8A.

3.3. Strain within the osteocyte:

The strain experienced in the representative osteocyte cell membranes is visible in the contour plots in Fig. 2D and Fig. 5. The strain distribution in the representative and idealised osteocytes is shown in Fig. 8B. While cellular strains greater than 10,000 με were predicted in discrete areas of the models most of the cell membranes, approximately 81-98% (σ = 90.1 ± 6.1%) were strained by less than 1,000 με.

4. DISCUSSION

This study develops the first fluid-structure interaction models to investigate the complex interaction between the solid and fluid phases within the osteocyte environment in vivo. These models simulate interstitial fluid flow arising from mechanical deformation of the bone matrix and pressure gradients under loading conditions representative of vigorous physical activity. This study allows the investigation of the velocities of the interstitial fluid, the shear stress imparted onto the surface of the cell and the resulting strain in the osteocyte cell processes and body. Such findings predict the mechanical stimuli to osteocytes under loading conditions known to stimulate an osteogenic response.

The modelling of a complex mechanical environment such as the lacunar-canalicular network necessitates a number of assumptions. The resolution of the images used to generate the models (0.1 μm), is amongst the highest obtainable on commercially available confocal microscopes. As the diameter of the canaliculus is ~100 nm, the most highly fluorescent canaliculi are more easily discernible and less fluorescent canaliculi are omitted during thresholding operations, resulting in fewer processes than exist in vivo. Although transmission electron microscopy (TEM) resolutions could overcome this issue, obtaining the full 3D information of an osteocyte through serial TEM is unfeasible due to the small size of ultrathin slices required (~9 nm) relative to the size of an osteocyte environment (~50 μm). It must be noted that, as the greatest stimulation in our models occurs in the canaliculi, we expect that inclusion of more canaliculi, as are present in vivo, would result in even greater stimulation of the osteocyte at these levels of loading.

For the purposes of this study the ECM and osteocyte materials were assumed to be linear elastic isotropic materials, with cell properties assigned from values observed in cultured bone cells (Sugawara et al. 2008) and the mesh-like pericellular matrix was not included. The viscoelastic nature of the cell was neglected as physiological loading of bones occurs at a frequency of approximately 1 Hz, well below the relaxation time of 41.5 s measured in bone

cells (Appelman et al. 2011; Darling et al. 2008). Furthermore the inherent anisotropy of the cell was neglected in this study in order to focus on the multi-physics effects of loading-induced fluid flow. However we have investigated the effect of transverse isotropy previously through a finite element approach and reported 3.7-12.2% greater strain transfer to the osteocyte when compared to isotropic cells (Verbruggen et al. 2012). Thus the stimulation predicted in the current study would be enhanced with the inclusion of an active actin cytoskeleton, which has been shown to result in higher reaction forces and increased tension inside the cells (Dowling et al. 2012; McGarry 2009; McGarry et al. 2009; Ofek et al. 2010; Ronan et al. 2012). As the fluid dynamics of the interstitial fluid are not well understood, laminar uni-directional flow was assumed based on studies of the nano-scale dimensions of the canalicular channels (Anderson et al. 2005; Cheng and Giordano 2002). Realistically dynamic flow and cyclic loading conditions occur in vivo, and experimental studies suggest that shear strain rate on the cell membrane is an important mechanical factor in bone mechanobiology (Bacabac et al. 2004; Fritton et al. 2000; Goldstein et al. 1991; Lanyon and Rubin 1984). The incorporation of dynamic physiological boundary conditions in future studies, along with an active actin cytoskeleton, would further amplify the stimulus observed in this study and provide more realistic simulations of osteocyte mechanobiology in vivo.

Mechanical loading in bone occurs on the whole organ level, with compression and tension occurring on opposite regions in the bone. This global loading generates a much higher pressure gradient than across the region of a single cell, driving fluid from one side of the organ to the other and resulting in gradients as large as the 300 Pa gradient applied in this study (Manfredini et al. 1999; Steck et al. 2003). A recent computational study has predicted pressure gradients within individual canaliculi as high as 1 Pa/nm, the equivalent of an approximately 800 Pa pressure gradient along the length of a single canaliculus (Kamioka et al. 2012). While we have represented this global loading as a localised pressure gradient in

this study we anticipate that an anatomically accurate lacunar-canalicular network, employing a multi-scale modelling approach to derive the boundary conditions across multiple scales (Vaughan et al. 2012), may elucidate the pressure gradient induced across individual osteocytes.

While previous researchers have employed CFD techniques to predict fluid flow in the lacunar-canalicular system (Anderson et al. 2005; Anderson and Knothe Tate 2008; Kamioka et al. 2012), this study provides the first investigation of the effect of bone matrix deformation on flow of interstitial fluid, and subsequent deformation of the osteocyte cell membranes in a fully three-dimensional anatomically-accurate osteocyte environment. While FSI modelling has recently been applied to model individual cells under in vitro conditions (Vaughan et al. 2013) and to investigate the mechanics of the bone marrow cavity (Birmingham et al. 2012a), these techniques have not yet been applied to the in vivo environment of individual bone cells. We report here that average velocities of approximately 60.5 μm/s occur within the pericellular space, which are similar to those observed in experimental fluorescein tracer studies (~60 μm/s) (Price et al. 2011).

Examination of the strain distribution in the osteocyte resulting from loading-induced fluid flow shows that strain levels are much lower than 10,000 με, which in vitro substrate strain studies have shown is required to stimulate osteoblast and osteocyte activity (Burger and Veldhuijzen 1993; You et al. 2000). Indeed most of the cell volumes (81-98%) experience strains below 1,000 με, an order of magnitude lower than that required for an osteogenic response. This implies that mechanically-driven fluid flow alone in the canalicular system is not sufficient to generate a strain-related osteogenic response. In a previous study we predicted strain stimuli of 23-26,000 με by a solid mechanics finite element approach using these representative models, assuming that the PCM was a solid continuum (Verbruggen et al. 2012). This glycocalyx matrix has been shown to reduce permeability by

an order of magnitude (Anderson et al. 2008), resisting flow and inducing strain in the osteocyte cell membrane (Goulet et al. 2009; Gururaja et al. 2005; Sansalone et al. 2012). Thus the inclusion of such a matrix would likely reduce the magnitudes of fluid velocity and the resulting shear stresses. However the presence of tethering elements in the PCM would also likely result in an increase in strain transfer to the osteocyte predicted here, as has been demonstrated analytically (Han et al. 2004; Wang et al. 2007; You et al. 2001; Zeng et al. 1994). The models in the current study do not include tethering elements as these are too small (~0.5 μm long) to be accurately captured at confocal resolutions, while serial TEM imaging of an entire osteocyte with tethering elements would prove extremely challenging due to the small size (~9 nm) of ultrathin sections required relative to the size on an osteocyte environment (~50 μm). We propose that the absence of these tethers of the peri-cellular matrix accounts for the low strain values predicted along the osteocyte membrane.

There has been much debate within the osteocyte community as to the effect of tethering elements in the pericellular matrix on flow through the lacunar-canalicular network, initially investigating mixing of solutes within the fluid under loading (Knothe Tate 2001; Wang et al. 2001). More recently the focus of this discussion has been whether the stimulus to the osteocyte is amplified through tethering elements (Han et al. 2004) or through ECM projections that disturb the flow or attach directly to the cell processes (Anderson and Knothe Tate 2008; Verbruggen et al. 2012; Wang et al. 2007). While tethering elements have not been included in this study, our results do show that variations in the canalicular geometry, particularly the ECM projections, act as shear stress stimulus amplifiers in the osteocyte geometry, similar to previous computational fluid dynamics (CFD) studies (Anderson and Knothe Tate 2008).

These projections have been shown to contact the cell process (Kamioka et al. 2012; McNamara et al. 2009) and also co-localize with areas of β3 integrin staining (McNamara et

al. 2009). β3 integrin is a key protein in αVβ3 focal adhesions which have been implicated in matrix invasive and attachment processes (Chatterjee and Chatterjee 2001; Huang et al. 2000) between bone surfaces and osteoclasts (Clover et al. 1992; Engleman et al. 1997; Horton et al. 1991). Additionally, osteopontin is a β3 ligand (Horton et al. 1991; Ross et al. 1993), which is present in abundance along the canalicular wall (Devoll et al. 1997; McKee and Nanci 1996; Noda et al. 2003; Sodek and McKee 2000). This evidence strongly suggests that these punctate represent a form of focal adhesion between the osteocyte and the canalicular wall. These attachments would result in both increased strain transfer from the ECM to the osteocyte (Verbruggen et al. 2012; Wang et al. 2007), and increased shear stress stimulus to the osteocyte through disrupted fluid flow as seen previously (Anderson and Knothe Tate 2008) and predicted in the current study.

The shear stimuli predicted here fall within the range of 0.1-2.2 Pa, which has been shown in cell culture studies of osteoblastic cells to result in increased nitric oxide (NO), prostaglandin (PGE_2) and osteopontin production, biochemicals known to play a key role in the osteogenic (bone forming) response of osteoblasts (Bacabac et al. 2004; Bakker et al. 2001; Owan et al. 1997; Smalt et al. 1997). Furthermore in vitro studies have also shown increases in intracellular calcium (Ca^{2+}), an important bone mechanotransduction signalling factor, at shear stress levels of 2 Pa (You et al. 2000). Therefore our findings suggest that under global loading conditions representative of vigorous activity (3000 με), the individual osteocyte is sufficiently stimulated to produce biochemical signals for bone formation.

Interestingly our results predict shear stresses on the cell processes in all models that are within the range of 0.8-3 Pa predicted analytically to occur in vivo (Han et al. 2004; Weinbaum et al. 1994; You et al. 2001; Zeng et al. 1994), and are similar to values (~5 Pa) suggested by tracer studies (Price et al. 2011). Our study predicts highly variable fluid

velocities and shear stresses within the canaliculi, with inhomogeneous flow patterns occurring similar to those recently predicted using detailed fluid mechanics models of individual canaliculi (Kamioka et al. 2012). However our study predicts that both the maximum velocities and highest shear stress levels were predicted to occur within the canaliculi, and that such stimuli were not predicted surrounding the cell body. Furthermore it was found that the inclusion of ECM projections along the wall of the canaliculi in idealised models resulted in an increase of 152% in shear stress stimulus to the cell. These stimuli were further amplified around these localised projections in representative osteocytes, with increases of 203-300% experienced. This is in agreement with previous finite element and fluid mechanics studies which found that the geometry of the canaliculi can greatly affect the stimulus experienced by the osteocyte cell process (Anderson et al. 2005; Anderson and Knothe Tate 2008; Kamioka et al. 2012; Verbruggen et al. 2012). The current study corroborates this evidence showing that under physiological loading conditions, representative of the in vivo multi-physics environment, interstitial fluid velocities and shear stresses that are both significantly greater in the canaliculi than around the osteocyte cell body. Furthermore, this is the first computational study to explore the effects of both the complex interplay between fluid and solid mechanics and the intricate architecture of the lacunar-canalicular network on osteocyte mechanobiology.

The mechanical stimulation within canaliculi is particularly interesting as focal adhesion complexes are localised in osteocytic processes (Kamioka et al. 2006; McNamara et al. 2009; Vatsa et al. 2008) and have been predicted to cause strain concentrations on osteocyte processes by analytical and fluid mechanics modelling approaches (Verbruggen et al. 2012; Wang et al. 2007). Previous cell culture studies have shown that the cell process contains more concentrated and highly organised actin filaments (Tanaka-Kamioka et al. 1998), and for these reasons the cell process is believed to be the most mechanosensitive area of the

osteocyte (Adachi et al. 2009). Our findings show that the canaliculus is a more mechanically active region of the lacunar-canalicular network than the lacuna, with the cell processes exposed to the greatest velocities and shear stimuli. Therefore these findings support the hypothesis that osteocyte cell processes in the canaliculi play an important role in osteocyte mechanobiology (Adachi et al. 2009).

5. CONCLUSIONS

In this study we report that fluid-structure interaction models of the osteocyte mechanical environment under global loading conditions representative of vigorous activity predict maximum shear stress (~11 Pa) stimulus and average fluid velocities (~60.5 μm/s) at the level of the individual osteocyte. Furthermore we observe that the highest stimuli occur in the canaliculi about the osteocytic process, reinforcing the theory that this is the most mechanically active and mechanosensitive region of the osteocyte. These are the first computational models to simulate the complex multi-physics mechanical environment of the osteocyte in vivo and to incorporate the complex 3D lacunar-canalicular architecture, providing a deeper understanding of osteocyte mechanobiology.

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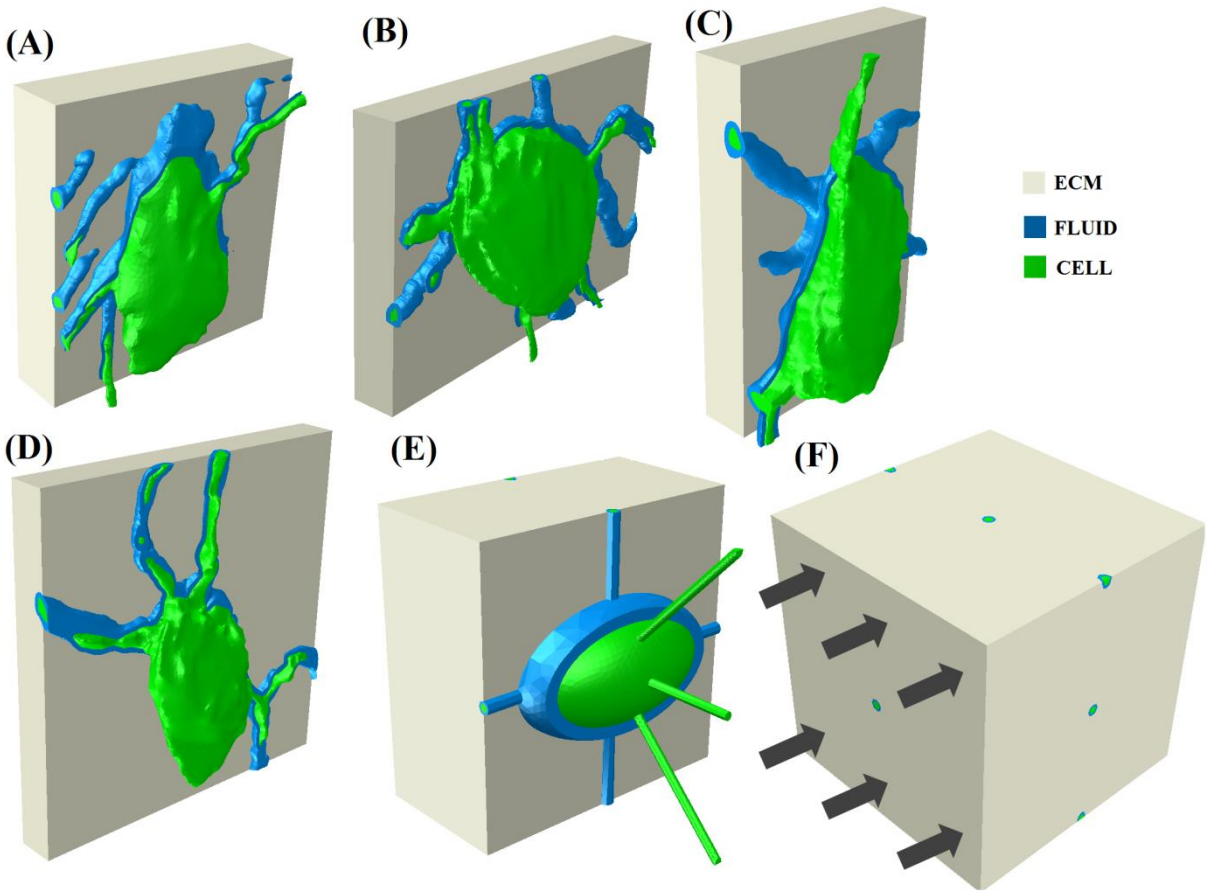

Fig. 1 Composite images of the three components of the representative models (A-D) and the idealised model (E). The face on which loading and inlet pressure conditions are applied is also shown (F).In each model the solid extracellular bone matrix (grey) is cut back to reveal the fluid-filled pericellular space (blue), which is in turn cut back to reveal the solid osteocyte beneath (green).

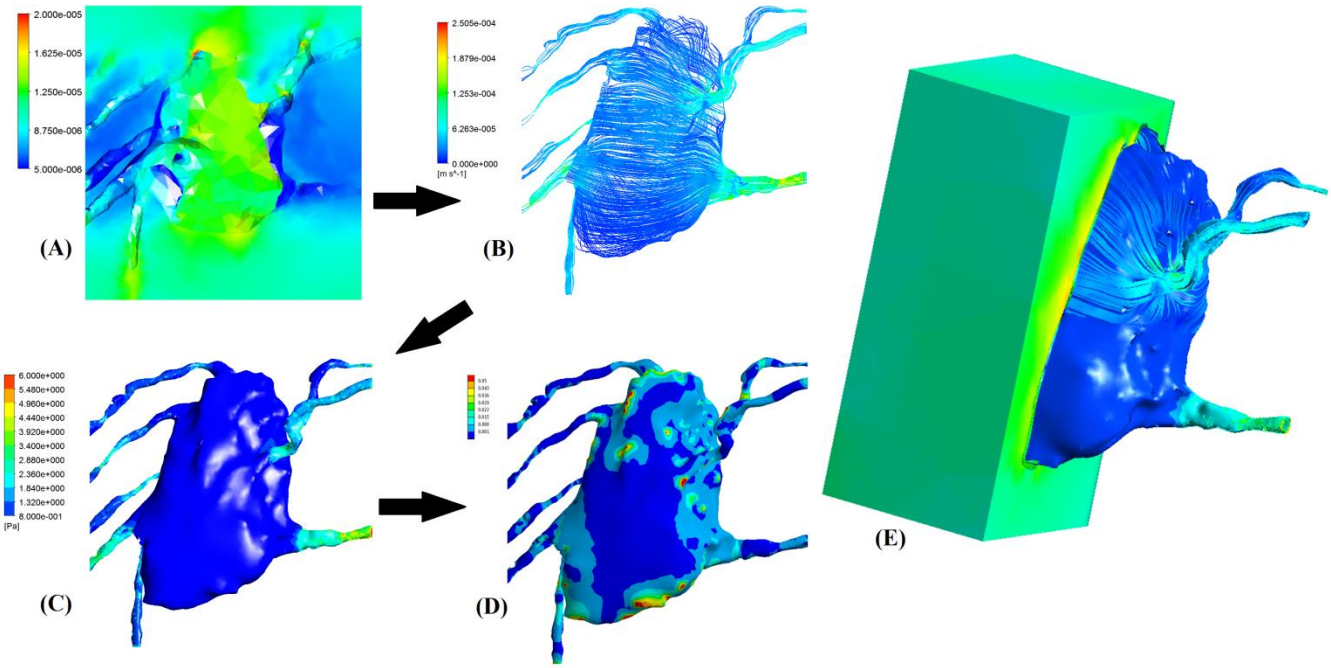

Fig. 2 (A) Strain distribution in the bone matrix around a representative osteocyte, (B) streamlines of the fluid flow within the pericellular space around the osteocyte, (C) shear stress imparted on the cell membrane by the fluid flow and (D) the resulting strain within the osteocyte. A composite image of these contour plots is also shown (E).

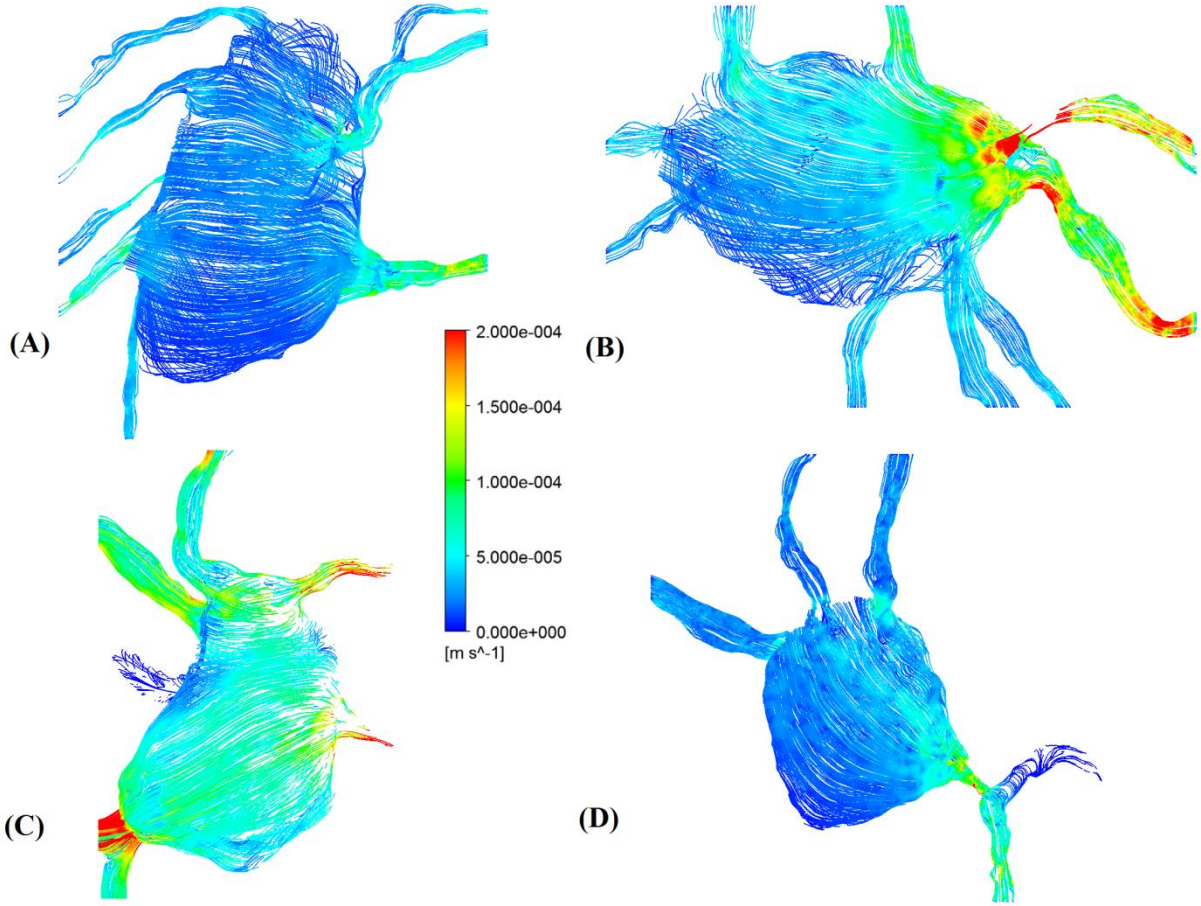

Fig. 3 Images showing velocity streamlines in pericellular space surrounding representative osteocytes

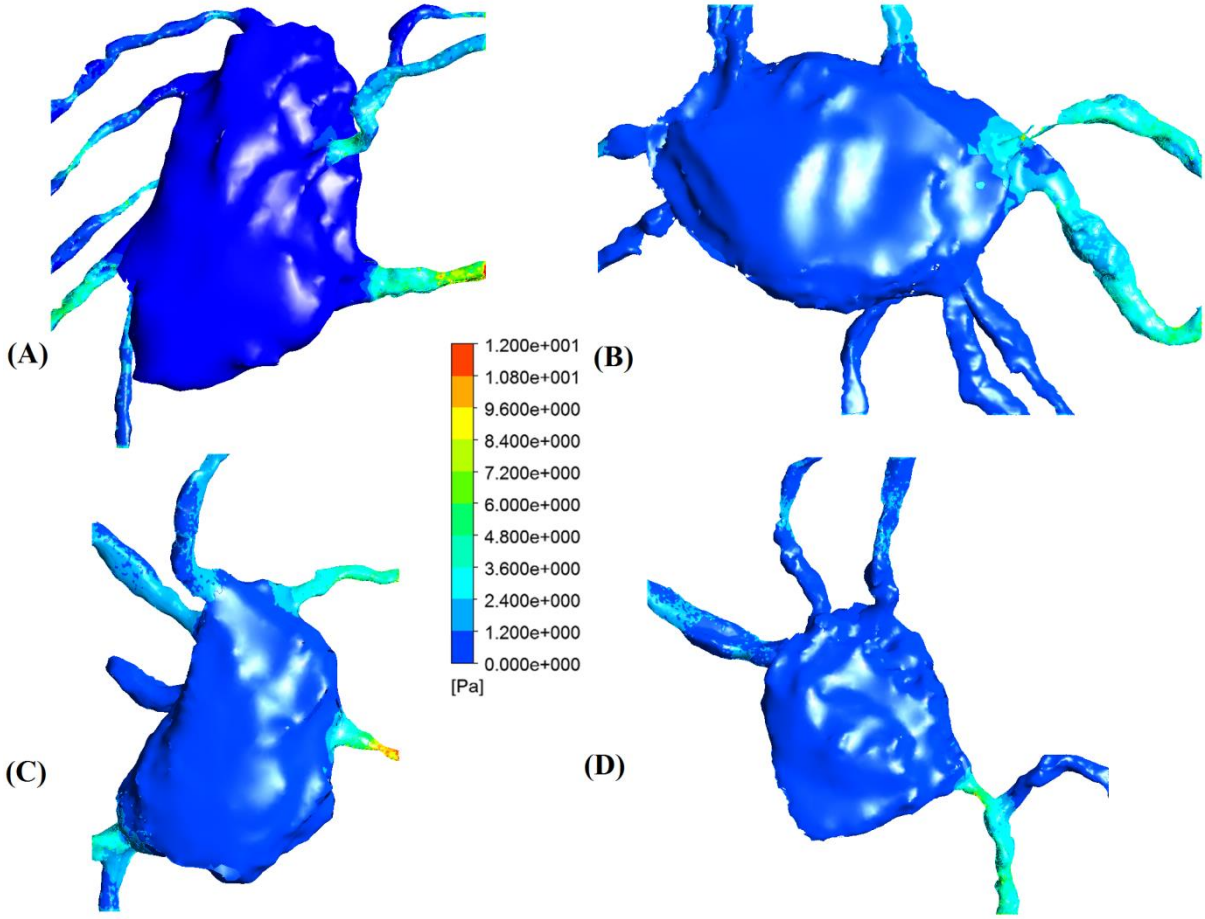

Fig. 4 Images showing contour plots of shear stress on representative osteocyte cell membranes.

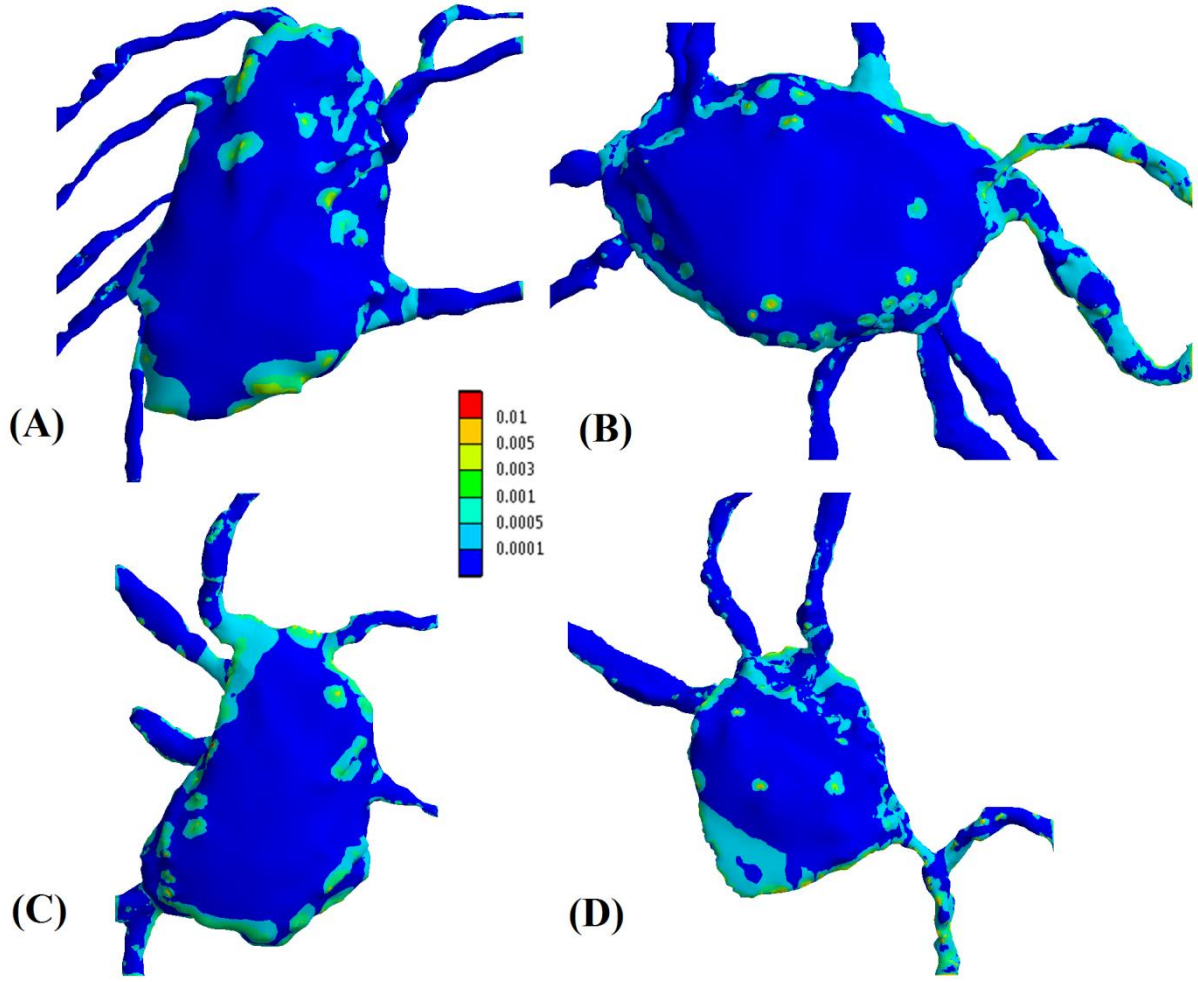

Fig. 5 Contour plots of maximum principal strain distributions in representative osteocyte cell membranes

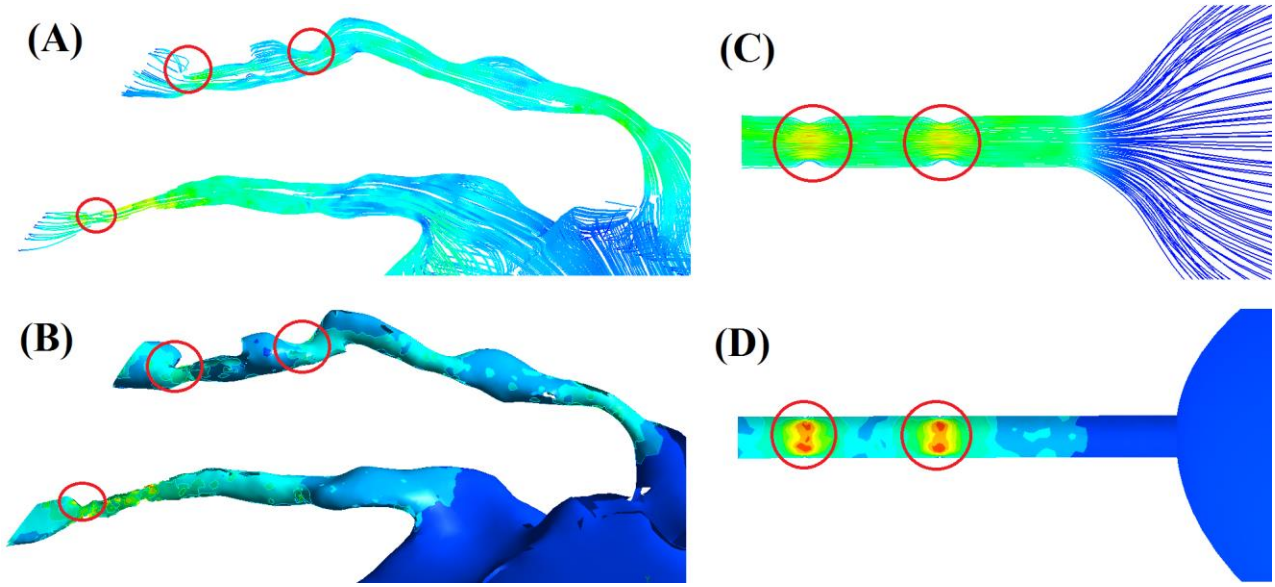

Fig. 6 Contour plots showing the stimulus amplification effect within the canaliculi, with the effect of the ECM projections clearly visible on (A) velocity and (B) shear stress in a representative osteocyte, and similarly on (C) velocity and (D) shear stress in an idealised model.

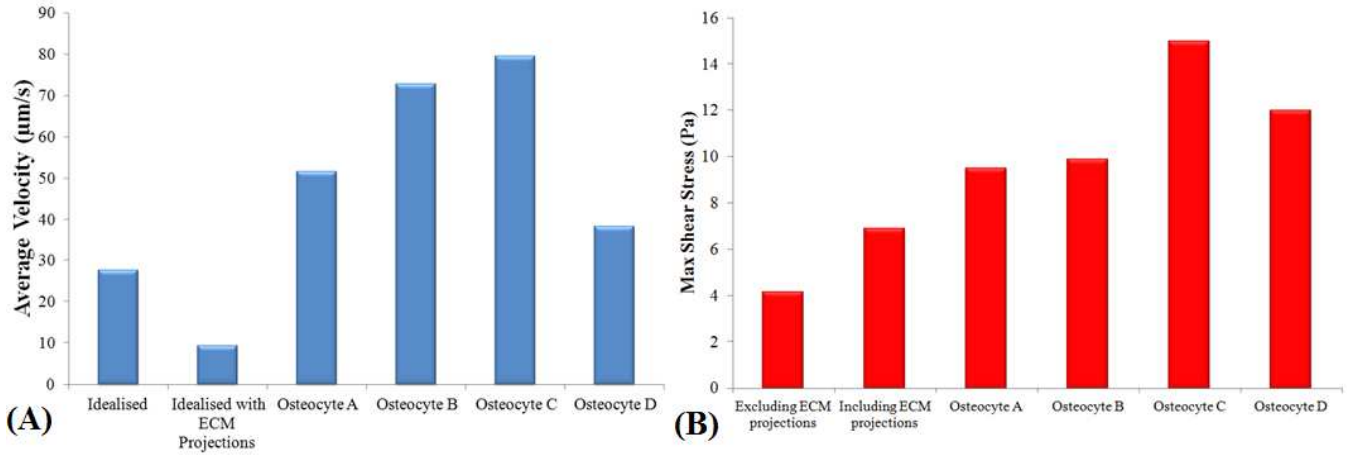

Fig. 7 Average interstitial fluid velocities within the pericellular space in osteocyte models (A) and the resulting maximum shear stresses on the osteocyte cell membranes (B).

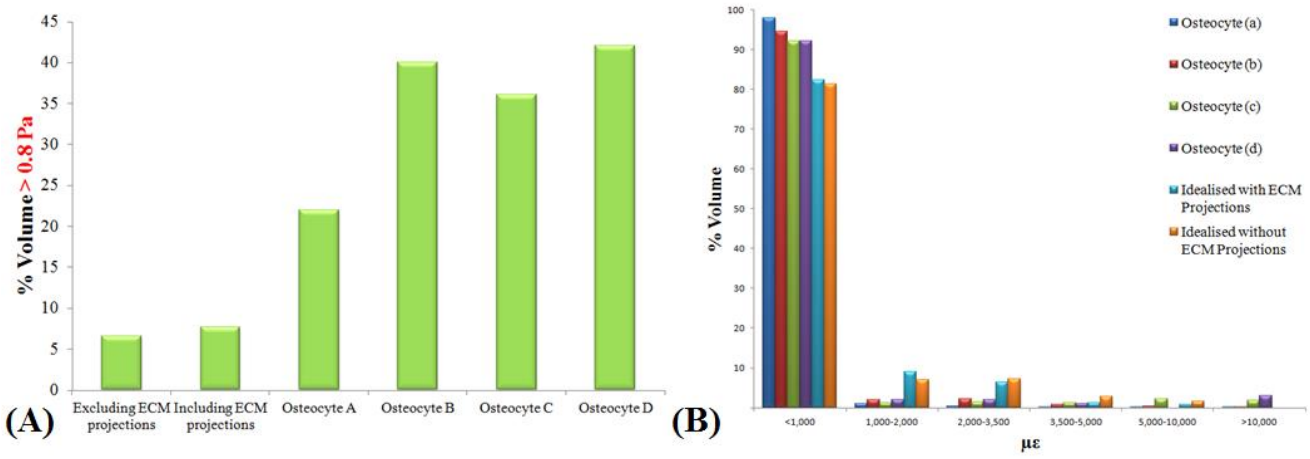

Fig. 8 The percentage of the osteocyte models stimulated above 0.8 Pa (A) and the strain distribution in the osteocytes arising from the mechanically-driven fluid flow (B).