Published in final edited form as:

J Biomech. 2014 June 27; 47(9): 2211–2217. doi:10.1016/j.jbiomech.2014.04.004.

Primary Cilium Mechanotransduction of Tensile Strain in 3D Culture: Finite Element Analyses of Strain Amplification Caused by 10% Tensile Strain Applied to a Primary Cilium Embedded in a Collagen Matrix

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Abstract

Human adipose-derived stem cells (hASC) exhibit multilineage differentiation potential with lineage specification that is dictated by both the chemical and mechanical stimuli to which they are exposed. We have previously shown that 10% cyclic tensile strain increases hASC osteogenesis and cell-mediated calcium accretion. We have also recently shown that primary cilia are present on hASC and that chemically-induced lineage specification of hASC concurrently results in length and conformation changes of the primary cilia. Further, we have observed cilia length changes on hASC cultured within a collagen I gel in response to 10% cyclic tensile strain. We therefore hypothesize that primary cilia may play a key mechanotransduction role for hASC exposed to tensile strain. The goal of this study was to use finite element analysis (FEA) to determine strains occurring within the ciliary membrane in response to 10% tensile strain applied parallel, or perpendicular, to cilia orientation. To elucidate the mechanical environment experienced by the cilium, several lengths were modeled and evaluated based on cilia lengths measured on hASC grown under varied culture conditions. Principal tensile strains in both hASC and ciliary membranes were calculated using FEA, and the magnitude and location of maximum principal tensile strain determined. We found that maximum principal tensile strain was concentrated at the base of the cilium. In the linear elastic model, applying strain perpendicular to the cilium resulted in maximum strains within the ciliary membrane from 150 to 200%, while applying strain parallel to the cilium resulted in much higher strains, approximately 400%. In the hyperelastic model, applying strain perpendicular to the cilium resulted in maximum strains within the ciliary membrane around 30%, while applying strain parallel to the cilium resulted in much higher strains ranging from 50% to 70%. Interestingly, FEA results indicated that primary cilium

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length was not directly related to ciliary membrane strain. Rather, it appears that cilium orientation may be more important than cilium length in determining sensitivity of hASC to tensile strain. This is the first study to model the effects of tensile strain on the primary cilium and provides newfound insight into the potential role of the primary cilium as a mechanosensor, particularly in tensile strain and potentially a multitude of other mechanical stimuli beyond fluid shear.

Keywords

Primary Cilia; Finite Element Analysis; Mechanotransduction; Tensile Strain; Ciliary Membrane; Mechanobiology; Ciliary Pocket; Adipose derived stem cells

Introduction

Primary cilia are organelles found in nearly every cell in the body. They are non-motile cilia structures and cells typically have one primary cilium at some point during the cell cycle (Singla and Reiter, 2006). Primary cilia have predominantly been studied for their role in polycystic kidney disease, where they have been shown to be vital in the detection of fluid shear stress (Winyard and Jenkins, 2011). However, they have also been shown to play a role in bone formation. It has been reported that they are critical for embryonic skeletal development in mice and zebrafish (Haycraft and Serra, 2008). Mutations in PKD1, the gene that codes for the primary cilia protein polycystin 1, have been shown to cause decreased skeletogenesis in embryonic mice (Xiao and Quarles, 2010). Further, primary cilia have been proposed as a mechanism for detection of fluid shear by osteocytes, although this was not empirically confirmed (Temiyasathit and Jacobs, 2010). The primary cilium is also considered to be important in osteogenic lineage specification of mesenchymal stem cells (MSC) in response to oscillatory fluid flow (Hoey et al., 2012; Tummala et al., 2010). Therefore, primary cilia comprise a growing area of investigation in bone tissue engineering.

Primary cilia have also been studied in other cell types. We recently reported their critical role in lineage specification of human adipose-derived stem cells (hASC) (Bodle et al., 2013) which can differentiate into musculoskeletal tissue similarly to MSC (Mauney et al., 2007; Ugarte et al., 2003; Weinzierl et al., 2006). We and others have previously shown that hASC exhibit mechanosensitivity with changes in proliferation and lineage specification in response to many types of mechanical stimuli (Charoenpanich et al., 2011; Hanson et al., 2009; McCullen et al., 2010; Park et al., 2011; Puetzer et al., 2013; Schatti et al., 2011; Wall et al., 2007). In particular, cyclic tensile strain, whether applied to hASC in monolayer (two-dimensional) or three-dimensional collagen culture, promotes hASC osteogenesis and cell-mediated calcium accretion (Deiderichs et al., 2010; Hanson et al., 2009; Wall et al., 2007). Given the role of primary cilia in detecting fluid shear stress, we hypothesize that primary cilia are a potential mechanotransduction mechanism in hASC since cilia extending from the cell body have the potential to be deflected by the collagen gel of 3D culture. One potential mechanism for primary cilia mechanotransduction of tensile strain is through stretch activated calcium channels (Nauli et al., 2003). However, it has been reported that cilia mechanotransduction occurs independently of calcium ion flux (Malone et al., 2007). We have recently shown that expression of cilia-associated proteins PC1 and IFT88 affects

hASC osteogenesis and moreover, changes in lineage specification of hASC result in length and conformation changes of the primary cilia (Bodle et al., 2013). Additionally we have observed that hASC exposed to 10% cyclic tensile strain will exhibit primary cilia length changes (unpublished data), consistent with work performed in tendon explants (Gardner et al., 2011). We hypothesize that these length changes occur to allow hASC, and other cells exposed to tensile strain, to modulate their sensitivity to strains in the surrounding mechanical environment.

Several previous studies have modeled primary cilia (Downs et al., 2012; Rydholm et al., 2010; Schwartz et al., 1997), although only one has used finite element analysis (Rydholm et al., 2010). Rydholm et al. (2010) developed a mechanical model of the primary cilium exposed to shear stress using Comsol Multiphysics (Rydholm et al., 2010). The model was comprised of two different layers, one representing the underlying structure of the cilia, and the other the cellular membrane (Rydholm et al., 2010). They reported that application of fluid shear stress resulted in a stress concentration in the cell membrane near the base of the cilium (Rydholm et al., 2010). Since this region is also the location of stretch activated calcium channels, the authors concluded that primary cilia mechanotransduction is likely related to these channels. Other cilia models have modeled primary cilia as they deflect under fluid shear (Schwartz et al., 1997). However, to our knowledge no previous models have modeled the primary cilia in a tensile strain culture environment. We hypothesized that primary cilia length changes would yield differential stresses and strains within the cilium structure that are thus translated to the hASC while under tensile strain; comprising a mechanosensory role for the hASC primary cilium and proposing a mechanical mechanism for the cilia length change phenomenon observed in culture.

Methods

Three idealized finite element models of a primary cilium extending from the cell membrane of an hASC were created using 3D structural mechanics in Comsol Multiphysics (Version 3.4a, Burlington, MA). Each model was generated using different cilium lengths acquired from empirical data of cilium lengths derived from immunofluorescently stained images of hASC. Standard fixation and immunofluorescent staining methods were used to generate the images. Briefly, hASC were fixed in 10% formalin for 20 minutes and then were permeabilized in 0.2% Triton-X-100 and 0.5% BSA in PBS. A primary mouse antibody against -acetylated tubulin (Sigma) with a chicken anti-mouse secondary and IFT88 with a donkey anti-goat (Alexafluor 594, Invitrogen) were used to visualize the cilium structure in 3D collagen I culture (**Figure 1A**). In both 2D and 3D culture images, DAPI was used to identify the cell nucleus. In 2D culture images, phalloidin 594 was used to visualize the actin cytoskeleton to establish cilium orientation with respect to the long axis of the cell body and the axis of strain (**Figure 1B**). Length data were derived from hASC cultured on collagen I-coated silicone membranes in 2D culture exposed to 10% cyclic tensile strain at 1Hz for 4 hours/day. The cells were cultured in complete growth medium (CGM), containing Eagle's Minimum Essential Medium, alpha-modified supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin; osteogenic differentiation medium (ODM), made of CGM and 50 µM ascorbic acid, 0.1 µM dexamethasone, and 10 mM -glycerolphosphate; or adipogenic differentiation medium

(ADM), CGM supplemented with 1 mM dexamethasone, 5mg/mL insulin, 100 mM indomethacin, and 500 mM isobutylmethylxanthine; for up to 72 hours to acquire representative lineage-specific cilia parameters (**Table 1**). ImageJ was used to measure the length and orientation of primary cilia. Over 400 cells were measured for each condition to generate average length values. Further, to inform our idealized 3D model, observations of primary cilia orientation and length on hASC cilia in 3D collagen I culture cultured with CGM and ODM under 10% cyclic tensile strain were also incorporated into the model. The hASC cultured in 3D were similarly stained and visualized using immunofluorescence.

Linear Elastic Model

Combining both 2D and 3D empirical observations, an idealized model comprised of a primary cilium and ciliary membrane attached to a $5\ \mu\text{m}^2$ segment of cell membrane where the cilium extends into a $5\ \mu\text{m}^3$ block of collagen gel (scaffold used for 3D cell culture) was created (**Figure 2A**) using Comsol Version 3.4a Structural Mechanics Module. The cilium was modeled as a continuous elastic beam extending $0.5\ \mu\text{m}$ into the cell body and extending out of the cell body at each of the following lengths dependent on culture medium conditions: (1) $3.05\ \mu\text{m}$ (complete growth medium (CGM) culture), (2) $2.72\ \mu\text{m}$ (osteogenic differentiation medium (ODM) culture) or (3) $3.90\ \mu\text{m}$ (adipogenic differentiation medium (ADM) culture). These numbers were calculated from the average cilia length for each culture condition and do not account for the variance in cilia length within each culture condition. The primary cilium was prescribed a radius of 100 nm (Molla-Herman et al., 2010) and a Young's modulus of 178 kPa (Huang et al., 2009). It was modeled as a solid cylinder of radius 95 nm coated with a 5nm thick ciliary membrane. This ciliary membrane was also connected to the cell membrane that extended in a $5\ \mu\text{m}$ by $5\ \mu\text{m}$ by 5nm thick square orthogonal to the cilium. The ciliary and cell membrane was prescribed a Young's Modulus of 1 kPa (Hochmuth et al., 1973). These two membranes were contiguous and modeled as a linear elastic material. A $5\ \mu\text{m}^3$ cube of collagen gel was placed over the primary cilium and the gel modeled as a homogeneous, linear elastic material with a Young's modulus of 17 kPa (Roeder et al., 2002). The collagen gel was modeled as fully bonded to both the cellular membrane and the ciliary membrane. Each model was evaluated under two strain conditions, 10% strain perpendicular to the primary cilium and 10% strain parallel to the primary cilium (**Figure 2B, C**). To apply 10% uniaxial tensile strain in a parallel direction, a $1780\ \text{N/m}^2$ load was applied to the two faces of the collagen cube orthogonal to the x-direction in the x and $-x$ directions. To apply 10% uniaxial tensile strain in the perpendicular direction, a $1780\ \text{N/m}^2$ load was applied to the two faces of the collagen cube orthogonal to the z-direction in the z and $-z$ direction. 3D graphs were generated of the first principal tensile strain in the membrane in each modeled condition. Both the magnitude and location of maximum strain of the cell membrane was determined for each condition.

Elastoplastic Model

A second set of three models were then created using the Structural Mechanics Module to include an elasto-plastic material for the cell and ciliary membrane. The models were identical to the linear elastic model described above except that the cell membrane was modeled as a Neo-Hookean elasto-plastic material with Young's Modulus of 1 kPa (Hochmuth et al., 1973), shear modulus of 30 MPa and bulk modulus of 16.7 MPa (Bausch

et al., 1998). In this method, the 10% tensile strain applied perpendicular to the primary cilium was applied by holding the left face of the cube orthogonal to the x-direction fixed while applying a 0.5 μm displacement to the other face orthogonal to the x-direction (**Figure 3A**). Ten percent (10%) strain parallel to the primary cilium was applied by holding the top face of the cube orthogonal to the z-direction fixed while applying a $-0.5 \mu\text{m}$ displacement to the bottom face orthogonal to the z-direction (**Figure 3B**). 3D graphs were generated of the first principal tensile strain in the membrane in each modeled condition. Both the magnitude and location of maximum principal tensile strain of the cell membrane were determined for each condition.

Results

To determine how tensile strain was transferred from the 3D collagen culture environment to the primary cilia and cells embedded within the collagen, principal tensile strains in the cell and ciliary membranes in response to an applied 10% strain of the collagen gel were calculated using FEA. Magnitudes and locations of highest principal tensile strain for both strain conditions (parallel or perpendicular to cilium) of each of three different primary cilium lengths were determined. All linear elastic models exhibited greatest magnitude of principal tensile strain in the ciliary membrane at the base of the cilium (**Figure 4**). All maximum principal tensile strains were within 50 nm of the base of the cilium. For the linear elastic ODM model with a cilium length of 2.72 μm , the maximum principal tensile strain was 380% for strain parallel to the cilium and 160% for strain perpendicular to the cilium (**Figure 5**). The linear elastic CGM model (3.05 μm) exhibited maximum principal tensile strains of 350% and 150% for parallel and perpendicular strain conditions, respectively. The linear elastic ADM model (3.90 μm) had maximum principal tensile strains of 340% and 170% for parallel and perpendicular strain conditions, respectively. Interestingly, primary cilium length did not directly correlate to membrane strain, nor did there appear to be a trend of increasing strain with increasing ciliary length.

Because these strains were too high to be accurately represented by a linear elastic model, three additional models were created incorporating a hyperelastic constitutive model for the cell membrane. These models also showed that the greatest principal tensile strain was exhibited at the base of the cilium (**Figure 6**). All maximum tensile strains were within 1 nm of the base of the cilium. As expected, the magnitude of principal tensile strains was much lower for the hyperelastic membrane model. For the ODM model, the maximum principal tensile strain was 68% for strain parallel to the cilium, and 30% for strain perpendicular to the primary cilium (**Figure 7**). The CGM model exhibited maximum principal tensile strains of 49% and 29% for parallel and perpendicular strain, respectively. For the ADM model, maximum principal tensile strains of 60% and 34% were shown for parallel and perpendicular strain, respectively. However, consistent with the linear elastic model, results using the hyperelastic model indicated no correlation between membrane strain and cilium length.

Discussion

Results of our finite element analyses indicated that tensile strain amplification was concentrated around the base of the primary cilium. These findings are consistent with previous investigations of primary cilium response to fluid shear concluding that fluid shear-induced strains are primarily concentrated at the base of the cilium (Rydholm et al., 2010). However, unlike the findings from those previous fluid shear models, our results indicate that primary cilium length does not appear to affect the amount of strain transmitted to the ciliary membrane for a primary cilium exposed to tensile strain as opposed to fluid shear. Based on our experimental observations that primary cilia exhibit length changes in response to culture under 10% cyclic tensile strain (**Table 1**), we expected that changes in primary cilium length might play a role in the ability of the primary cilium to detect these tensile strains. However, findings from this study indicate that the orientation of the primary cilium with respect to the tensile strain appear to be more important in detecting tensile strains than the length of the primary cilium. In our calculations of tensile strain, strain parallel to cilium orientation resulted in a greater strain amplification in the ciliary membrane, leading us to conclude that the primary cilium will have greater sensitivity to tensile strain applied parallel to cilium orientation than to that applied perpendicular to cilium orientation. Interestingly, a study by Lavagnino *et al.* that imaged and quantified cilia on tendon explants demonstrated a correlation between cilium deflection angle and tensile strain (Lavagnino et al., 2011). Taken together with our model's predictions, cilia orientation is likely a key mechanism of modulating the mechanosensitivity of the cilium structure. Further, their empirical observation in tendon, combined with our computational data suggests that orientation likely modulates the molecular mechanisms localized in the cilium. Although it does not disprove our theory that cilium length changes during differentiation to allow differences in mechanosensitivity between cell types, it does indicate that orientation, rather than length may be more directly involved in modulating cilia mechanosensitivity.

Given our findings of tensile strain amplification at the base of the cilium, future studies and computational models should evaluate refinement of the current model in the region around the cilium base. Primary cilia on many cell types have a feature at the base of the cilium known as the ciliary pocket. This pocket is a remnant of cellular membrane from ciliary growth, but is also the location for a number of endocytotic processes (Clement et al., 2013) and serves as an interface between the cilium and the actin cytoskeleton (Benmerah, 2013). The presence and structure of this ciliary pocket may be of importance in strain amplification in the primary cilium. Inclusion of cilia pocket architecture should be incorporated in future models.

Findings from this study lead to other exciting questions that could be addressed in future work. We have recently shown that primary cilia on hASC not only exhibit changes in length when hASC undergo differentiation, but also exhibit changes in conformation. Both primary cilia length and conformation alterations have also been observed on tenocytes in response to the mechanical environment (Gardner et al., 2011; Lavagnino et al., 2011). Future computational studies should expand upon these empirical findings to include analyses of primary cilia with varying shapes to determine the role of primary cilium shape in detecting strains in the surrounding environment. It is possible and indeed probable that

primary cilia that exhibit significant changes in conformation might be better able to detect strains in multiple directions.

Such computational investigations could be performed concurrently with *in vitro* investigations of ciliary changes to validate mechanically-induced molecular mechanisms. Since we suspect that hASC might be adapting ciliary length to better sense their mechanical environments, but have now found that cilia length might not be as sensitive to changes in applied tensile strain as cilia orientation, it is possible that cilium orientation is being modified in response to applied tensile strain. Studies of tenocytes have shown that primary cilia typically align in the direction of migration (Gardner et al., 2011). Measurements of primary cilia orientation with respect to the direction of applied strain could potentially be a method to determine if hASC are using the cilia to sense and respond to changes in direction of tensile strain.

In conclusion, this is the first study to computationally predict the principal tensile strains and localized strain profiles on primary cilia exposed to strain applied either perpendicular or parallel to the ciliary axoneme. The findings of this study suggest that the orientation of the cilium structure within a tensile strain environment has a large impact on strain distribution within the structure and thus may be a mode of modulating mechanically-induced cell processes. In the case of hASC, this may be involved in mechanically-enhanced differentiation and/or may have implications for lineage-specific mechanosensitivity. These new insights provide great advances to our understanding of the role of primary cilia in the detection of mechanical forces at the cellular level. Further, it indicates that primary cilia can play a key mechanosensory role in tissues that are not exposed to fluid shear.

Acknowledgments

This study was funded in part by an NIH NCRR grant 10KR51023 (E.G.L.), by NIH/NIBIB grant R03EB008790 (E.G.L.), and by NSF/CBET grant 1133427 (E.G.L.).

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Figure 1.
Primary cilia on hASC visualized by acetylated α -tubulin (green), in 3D collagen I culture with further staining for IFT88 (red) (**a**) and in 2D culture with further staining for actin (red) (**b**). DAPI = Nuclei (Blue). Scale bar represents 25 μ m.

Figure 2. Boundary conditions and geometry for linear elastic model. A) Primary cilium model shown with cell and ciliary membrane in purple. Collagen gel is shown in pink. B) Perpendicular strain is applied along the x-axis. C) Parallel strain is applied along the z-axis.

Figure 3. Boundary conditions and applied forces for the hyperelastic model. A) Perpendicular strain is applied along the x-axis by holding one face of the cube and displacing the opposite face. B) Parallel strain is applied along the x-axis by holding one face of the cube and displacing the opposite face.

Figure 4.

Representative images showing strain amplification at the cilium base in the linear elastic model. Principal tensile strain is shown in the cell and ciliary membrane for a primary cilium $3.05\mu\text{m}$ in length. Cilium strained perpendicular to cilium orientation (A, B) exhibited less membrane strain than cilium strained parallel to cilium orientation (C, D). Both directions of applied tensile strain result in strain concentrations at the cilium base.

Figure 5.

Maximum principal tensile strain in the ciliary membrane in the linear elastic model for cilium with 10% tensile strain applied to surrounding collagen gel either parallel or perpendicular to the cilium. Perpendicular strain induces maximum tensile strain in the range of 150-200% while parallel strain shows maximum strains between 300-400%. There is no apparent trend between cilium length and magnitude of maximum principal tensile strain. ODM = osteogenic differentiation medium; CGM = complete growth medium; ADM = adipogenic differentiation medium.

Figure 6.

Representative images showing strain amplification at the cilium base in the hyperelastic model. Principal tensile strain is shown in the cell and ciliary membrane for a primary cilium $3.05\mu\text{m}$ in length. Cilium strained perpendicular to cilium orientation (A, B) exhibited less membrane strain than cilium strained parallel to cilium orientation (C, D). Both directions of applied tensile strain result in strain concentrations at the cilium base.

Figure 7.

Maximum principal tensile strain in the ciliary membrane in the hyperelastic model for cilium with 10% tensile strain applied to surrounding collagen gel either parallel or perpendicular to the cilium. Perpendicular strain induces maximum principal tensile strains within the ciliary membrane around 30% while parallel strain induces maximum principal tensile strains within the ciliary membrane between 50-70%. There is no apparent trend between cilium length and magnitude of maximum principal tensile strain. ODM = osteogenic differentiation medium, 2.72 μm = primary cilium length used for ODM model; CGM = complete growth medium, 3.05 μm = primary cilium length used for CGM model; ADM = adipogenic differentiation medium, 3.90 μm = primary cilium length used for ADM model.

Table 1

Primary cilium lengths used in finite element models. Three different primary cilium lengths were based on empirical measurement of primary cilium lengths on human adipose derived stem cells (hASC) cultured in either complete growth medium (CGM), osteogenic differentiation medium (ODM) or adipogenic differentiation medium (ADM) in the presence of 10% cyclic tensile strain.

CGM	ODM	ADM
3.05 μm	2.72 μm	3.90 μm