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Computational Simulation of Applying Oscillatory Flow on a Stem Cell Using Fluid-Structure Interaction Method: Role of Primary Cilia and Cytoskeleton

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ABSTRACT: Load-induced fluid flow acts as a dominant biophysical signal for bone cell mechanotransduction in vivo. Oscillatory fluid flow has been used in bone tissue engineering strategies due to its similarity to the fluid dynamics within the human body. In this study, a fluid-structure interaction method was used to subject the mesenchymal cell to steady and oscillatory fluid flow. Three models were considered for a steady flow, including cytoplasm, nucleus, primary cilium, and cytoskeleton, to investigate the effects of cilium and cytoskeleton on cell mechanical responses (stress and strain). The fourth model, including cytoplasm, primary cilium, and cytoskeleton components has been considered to evaluate the stress and strain values created in the cell and its components in the oscillatory flow regime. The length and mechanical properties of the primary cilium (Young's modulus) were also varied to investigate cell responses. The results indicated that the presence of the cytoskeleton reduced the amount of stress experienced in the cell by about 35%. The presence of primary cilium, also, increased stress in the cell by about ten times in an oscillatory regime. The peak von Mises stress was 11.5 Pa in the oscillatory flow, which is three times greater than the level observed in the steady state condition. Moreover, the highest amount of strain occurred at the base of the cilium, indicating this component's importance in receiving and transmitting stress to other components. Our results revealed a direct relationship between the properties of the cilium and the stress and strain created in the cell. For a cilium with a length of 4 µm, the deflection at the tip of the cilium was 0.77 µm. This represented a 78% increase compared to a 10 µm cilium. This research can be a basis for future numerical studies in tissue engineering and improvements in the related experimental approaches.

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1- Introduction

In regenerative medicine, procedures that focus on developing and applying new treatments to repair bone defects created by either injury or disease are a rapidly field of interest [1]. Bone is a specialized hard tissue that provides structural support, protects critical internal organs, and maintains mineral hemostasis [2]. Therefore, it is necessary to regenerate bone problems such as osteonecrosis and osteoporosis [3]. Although bone graft techniques, such as autografts and allografts, are considered the gold standard in regenerative orthopedics, they can cause severe problems [4]. For instance, the higher risk of inflammation, immunogenic reactions, and disease transmission can be noted. Due to these limitations, bone tissue engineering (BTE) has been using an alternative strategy for regenerating damaged bone tissues [5, 6]. Mesenchymal stem cells (MSCs) are multipotent cells that can be isolated from a variety of tissues. In addition, MSCs have the ability to differentiate into various organs or tissues, such as bone [7, 8]. Recent experimental results demonstrate that shear stress, one of the dominant mechanical stimuli, can effectively enhance the osteogenesis and bone mineralization of MSCs. It is important to note that local mechanical stimuli facilitate biological cellular procedures at the cell level, such as osteogenic differentiation, migration, and proliferation [2].

In a study by Kongzu Hu et al. [9], fluid shear was applied at a rate of 1.2 Pa to induce osteogenic differentiation of bone marrow stem cells (MSCs). The study demonstrated that fluid shear stress can influence the differentiation fate of MSCs. It is well-accepted that oscillatory fluid flow (OFF) promotes the osteogenic differentiation of MSCs due to its similarity to the physiological pattern rather than unidirectional shear stress [10]. Another former study [11] investigated the effect of oscillatory shear stress magnitude, frequency, and duration on the osteogenic responses of MSCs. They found that the frequency of 2 Hz induces the most robust and reliable upregulation in osteogenic gene expression. Furthermore, in a recent study by Mohseni et al. [12], the effect of both vibration and oscillatory fluid flow on the cell components was investigated. The results showed that the maximum shear stress on the MSC was about 2.87 Pa, and a frequency of 30

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Hz was suggested to increase the level of strain. Therefore, the results demonstrate that using OFF can enhance stem cell differentiation.

The cytoskeleton plays a significant role in the differentiation of MSCs. For instance, actin filaments may act as a mediator in fluid shear stress-induced osteogenic differentiation. Several studies have reported that MSCs exhibit a flower shape during adipogenesis and a star shape during osteogenic lineage commitment [13]. The cytoskeleton plays a crucial role in determining the size, shape, and stiffness of the nucleus. Additionally, gene transcription and expression occur within the nucleus [14]. Although microtubules are not typically considered mechanical receptors, they do play a key role in various cellular activities such as vesicle transport, cilium formation, and cell polarity [15, 16]. In differentiated cells, the nucleus is approximately five to ten times stiffer than the surrounding cytoskeleton; due to its size and stiffness, the mechanical properties of the nucleus often dominate the overall cellular behavior during physiological deformation [17]. The primary cilium's significance in regulating mechanotransduction in human bone MSCs was indicated by initial findings, as it may act as a mediator [18]. In a fluid-structure interaction analysis by Ahmadian et al. [19], cilium, as an intracellular component, was examined under oscillatory flow. The results showed its greater sensitivity than the receptors in the cell membrane. In addition, when the cell was exposed to oscillatory fluid, the active ion channels experienced more stress than in the steady-state fluid flow.

Computational methods have been used in various fields of engineering, not only to reduce costs but also to improve our understanding of the mechanobiological responses for a wide range of scaffolds and target tissues in the field of bone tissue engineering [20, 21]. Theoretical models can be used to predict how the contributions of deformable intracellular components are integrated to determine the overall balance of mechanical force within the cell [22]. A computational study by Barrato et al. [23] has shown the transmission of force inside the cells to determine the practical cytoskeleton components of cellular responses. It has been investigated that each component has a different response; however, actin bundles along with microtubules are the main components that resist shear loads.

Oscillatory flow has been suggested by experimental studies. However, studies investigating the effects of oscillatory fluid flow on individual MSC components are less frequent than those investigating steady flow. To this end, in this study, a computational method was used to investigate the effect of oscillatory flow on both the cytoskeleton and primary cilium. Therefore, cellular components were stimulated separately in a steady state condition. Finally, a stem cell with its intracellular components, including cytoplasm, nucleus, primary cilium, and cytoskeleton components, was analyzed under oscillatory flow. Computational study of the mechanical responses of different cell components may help researchers in the field of tissue engineering to improve the experimental approaches by correctly interpreting the mechanical responses of MSCs under *in vitro* mechanical stimulations.

2- Materials and methods

The fluid domain considered in this study was a channel with dimensions of $50\times50\times40\ \mu m^3$, while the solid domain was represented by a single cell adhered to the bottom of the channel. In a steady fluid flow, three models with different cell components were considered: The first model, as the basic model, included cytoplasm and nucleus. The primary cilium was then added to the geometry in the second model. In the third model, we have considered the cytoplasm and the nucleus, covered by the actin cortex as well as the actin boundless and microtubules representing the cytoskeleton fibers. By using three different models, it was possible to investigate and compare the effects of cellular components on cellular responses. This study also parametrically varied the length of the primary cilium from 3 to 10 µm and also Young's modulus to evaluate their effects on cellular stimulations.

2- 1- Cell geometry and materials properties

Different components were considered for the 3D finite element model, including cytoplasm, nucleus, primary cilium, and the filaments of the cytoskeleton, which were actin and microtubules. To conduct a comparative study, three distinct geometries were generated. According to several experimental studies [24], stem cell was considered semi-ellipsoids with a major diameter of 20 µm and a minor diameter of 10 µm. The nucleus, located at the center of the stem cell, has an elliptical shape. Nucleus volume is one-third of the cytoplasm [25]. Figure 1(a) illustrates the first model, where the cytoplasm and the nucleus are included. In the second model, the primary cilium was added since it is a critical mediator in the mechanosensing process [26], where a cylinder with a diameter of 0.2 µm and a height of 4 µm was used to model it (Figure 1(b)) [27]. In the third model, apart from the nucleus, cytoplasm, and cilium, the cytoskeleton was considered, which contained a network of filamentous polymers [28] (Figure 1(c)). Therefore, cytoskeleton components, including 17 actin fibers and 14 microtubules in a star-shaped structure with a diameter of 12.5 µm and 0.24 µm, respectively, and actin cortex as a shell with a thickness of 0.2 µm were considered [29]. The adjacent cortex of actin has a higher mechanical property (i.e., Young's modulus and stiffness) than the cell membrane; therefore, in this study, the membrane has not been considered. In the current study, actin bundles, which were distributed throughout the cell, were connected to the cilium. In addition, intermediate filaments were ignored to reduce computational costs [30, 31]. The values of Young's modulus, Poisson's ratio, and density of all cell components were noted in Table 1. The solid domain in the model was assumed to be continuous, homogeneous, isotropic, and linearly elastic.

2- 2- Governing equations

In this study, we used continuity Eq. (1) and the conservation of momentum Eq. (2) [32, 33] as the governing

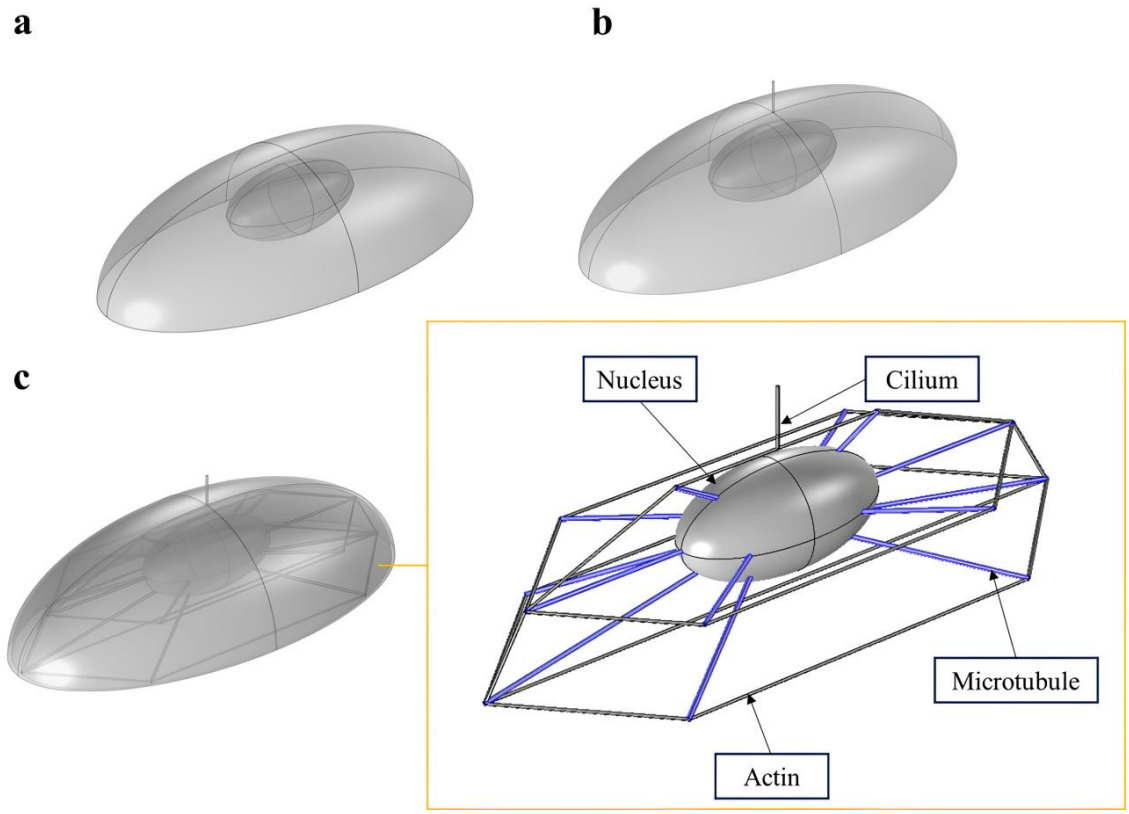

Fig. 1. The stem cell geometrical model. (a) The first MSC model includes cytoplasm and the nucleus. (b) The second MSC model includes the cytoplasm, the nucleus, and the primary cilium. (c) The third model includes the cytoplasm, the nucleus, the primary cilium, and cytoskeleton components which are actin filaments and microtubules.

Table 1. Mechanical properties of the intracellular components.

Material	Properties	Value	Unit	Reference
Cytoplasm	Young's modulus Poisson's ratio Density	15.4 0.49 930	kPa - kg/m^3	[25]
Nucleus	Young's modulus Poisson's ratio Density	11.9 0.49 1080	kPa - kg/m^3	[25]
Cilium	Young's modulus Poisson's ratio Density	178 0.33 1110	kPa - kg/m^3	[29]
Microtubule	Young's modulus Poisson's ratio Density	2000 0.3 990	GPa - kg/m^3	[29]
Actin	Young's modulus Poisson's ratio Density	340 0.3 870	kPa - kg/m^3	[29]
Actin cortex	Young's modulus Poisson's ratio Density	2 0.3 1050	kPa - kg/m^3	[29]

equations for an incompressible laminar flow:

$$\rho_f \nabla . u_f = 0 \tag{1}$$

$$\rho_f \left(\frac{\partial u_f}{\partial t} + (u_f - w) . \nabla u_f \right) = -\nabla P_f + \mu_f \nabla^2 u_f + \rho_f f \tag{2}$$

where u represents the fluid velocity vector, w is the mesh velocity vector, P is the fluid pressure, ρ represents the density of the medium, t is the time, μ represents the medium dynamic viscosity, and f is the body force per unit mass. In this study, for the solid domain, isotropic linear elastic properties were considered, which were calculated using Eq. (3) where d_s . shows the displacement of the solid domain, υ represents the Poisson's ratio, and σ is the solid stress tensor [3].

$$\rho_s \frac{\partial^2 d_s}{\partial t^2} - \nabla . \sigma_s = (\mathrm{I} + \nabla d_s) \upsilon_f \tag{3}$$

To have a two-way coupling solution, where the deformation of the fluid-solid interface deforms the fluid mesh, the Lagrangian-Eulerian method is required. Theelated governing equations (4-6) are shown to govern the interface between the two domains mentioned [34]:

$$u_i = \frac{\partial d_s}{\partial t} \tag{4}$$

$$\sigma_f . n = \Gamma . n \tag{5}$$

$$\Gamma = (\mathrm{PI} + \mu(\nabla u_f + (\nabla u_f)^T)) - \frac{2}{3}\mu(\nabla u_f) I \tag{6}$$

where u_i is the velocity of the wall at the interface between two domains and Γ is the transitional hydrodynamic stress from the fluid to the solid domain.

2- 3- Boundary conditions

According to Figure 2, a single cell through a cubic channel has been considered for this study, in which a fixed constraint boundary has been defined to prevent the cell from moving in response to the flow. Moreover, the fluid enters the cube through the left side and exits through the right side. The outer surface of the actin cortex and the primary cilium formed the fluid-solid interface where forces and displacements were transferred between the fluid and solid domains. To investigate the mechanical stimulation of an MSC under the fluid flow, a fully coupled FSI model was defined [35]. Two situations were modeled, including steady-state and oscillatory flow. In the steady state condition, a laminar flow regime was considered in the cube. The fluid was assumed to be water; hence, the density and dynamic viscosity were $\rho =$ 997 kg/m^3 and $\mu =$ 8.99e-4 kgms^{-1}, respectively [36]. A constant velocity profile, $u = 100\mu$ m/s, was set at the inlet, while a non-slip boundary condition was assumed for all cube walls. A zero-pressure boundary condition was imposed at the outlet [10]. In several experimental studies, MSCs were exposed to OFF at frequencies of 0.5 Hz, 1 Hz, and 2 Hz [11]. We have used a frequency of 1 Hz because it is the most common and reliable regime in osteogenic gene expression, and interestingly, most *in vitro* studies used a frequency of 1 Hz [37, 38]. Experimental studies have suggested the incompressible oscillatory flow with a frequency of 1 Hz that can be expressed by Eq. (7):

$$v = 30 + 300\pi \sin(2\pi f t) \tag{7}$$

in which v is the inlet velocity to generate shear stress on MSC surface, and f is the frequency. To study the oscillatory regime, the time step was set to 0.05 s in a time interval of 0–1 s.

2- 4- Solution method

Fluid-structure interaction and finite element methods have been used to solve the problem. A two-way FSI approach was used in this study to investigate the effect of both steady and oscillatory flow regimes on the cellular components using COMSOL Multiphysics 6.1 (Palo Alto, CA) by coupling the physics of laminar flow and solid mechanics. The two-way approach allows the interaction of forces from fluid to solid domains and displacement from solid to fluid domains to be fully coupled. The first three models, including the cytoplasm, nucleus, primary cilium, and cytoskeleton, were stimulated in a steady state condition separately, and the last model with all the mentioned components was studied in a time-dependent condition using PUMPMS and Paradiso solvers. In addressing a Multiphysics model, there are two methods available for solving the system of equations that define the solution: fully coupled and segregated. We employed the segregated method, which divided the problem into two or more separate steps. Each step represented an individual physics aspect, and these were solved sequentially within a single iteration, requiring less memory. Additionally, the relative value, which serves as one side of an inequality expression known as the convergence criterion, was set to 0.001. According to Figure 2 the FSI model includes an MSC as a solid deformable domain attached to the bottom plate of the cube. Due to the importance of precise results, such as stress and strain distributions in narrow areas, including

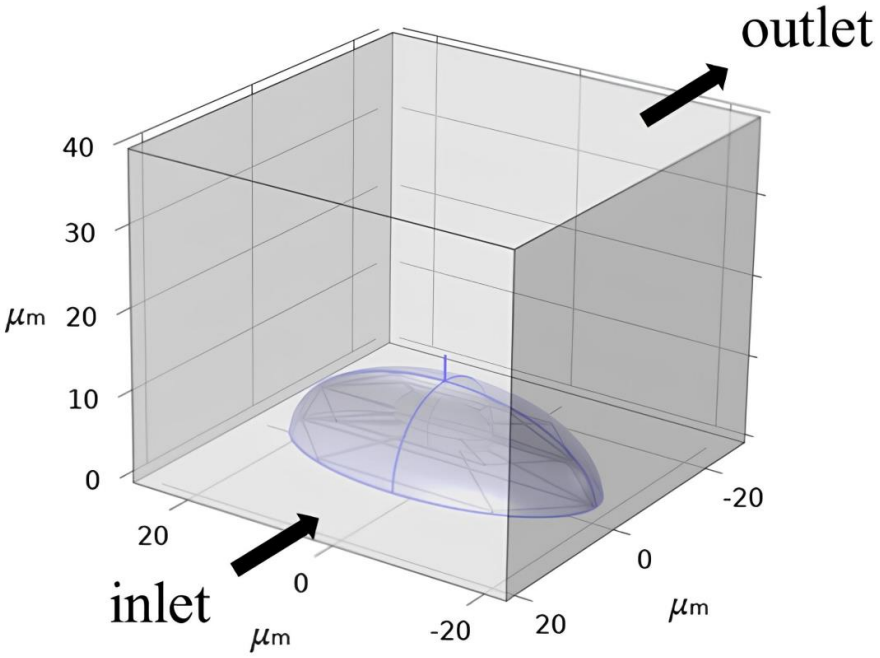

Fig. 2. Boundary conditions of the MSC inside a cubic bioreactor.

the junction of the cytoskeleton and the primary cilium, and the interface of microtubules and actin elements, a user-defined mesh was generated. The number of elements in either the solid or fluid domain has been reported as 297104 and 114742, respectively (Fig. 3(a), (b)). To check the mesh independence of the numerical results, the amount of maximum shear stress was calculated in six different steps, where the number of elements was considered to be 125896, 221987, 317998, 411846, 500320, and 605911. After four steps of increasing the number of elements, the differences in the magnitude of the maximum shear stress were found to be less than 5%. Therefore, the number of elements in the fourth step, including 411846 elements, was considered to report the results (Fig. 3 (c)).

3- Results

This study assessed the impact of steady and oscillatory flow on cellular components, aimed to determine the influence of cytoskeleton and primary cilium on the fate of MSCs *in vitro*.

3- 1- Steady state flow

The model of a single cell, which includes cytoplasm and nucleus, was used to predict the von Mises stress of a cell under steady-state fluid flow. According to Figure 4(a), the average von Mises stress on the cytoplasm surface ranged from 0.08-0.5 Pa, where the maximum and minimum values of von Mises stress were found in the side area and at the top of the cell, respectively. Figure 4(b), demonstrates the von Mises distribution on the nucleus, which ranged from 0.1 to 0.14 Pa under steady-state fluid flow. The results for this parameter indicate that the nucleus experienced approximately five times less stress than the bottom part of the cell. When the primary cilium was added to the cytoplasm and nucleus, the average amount of von Mises stress increased to a range of 0.03 to 5.23 Pa, which was approximately ten times higher than in the first model. As shown in Figure 4(c), the maximum von Mises stress occurred where the cilium and the cell were attached. In this model, the von Mises stress experienced by the nucleus was 0.18 Pa, which is approximately 28% higher than in the first model Figure 4(d)). Hence, this shows the importance of primary cilia being involved in mediating bone mechanotransduction. By considering the components of the cytoskeleton, the maximum von Mises stress was reduced by 35% as compared to the last model. Therefore, cytoplasm stress was reported from 0.03 to 3.40 Pa. As shown in Figure 4(e), the maximum stress was observed at the junction of the microtubules and nucleus in the third model, while the minimum was on the cell surface, so cytoplasm stress values were transduced to the nucleus. Figure 4(f) shows the distribution of von Mises stress on the nucleus surface, which is attached to the microtubules. The maximum stress was 3.4 Pa, three times greater than the last model. Based on the results of the three models, the study investigated how different cellular components contribute to the increase or decrease of the maximum von Mises stress and strain under

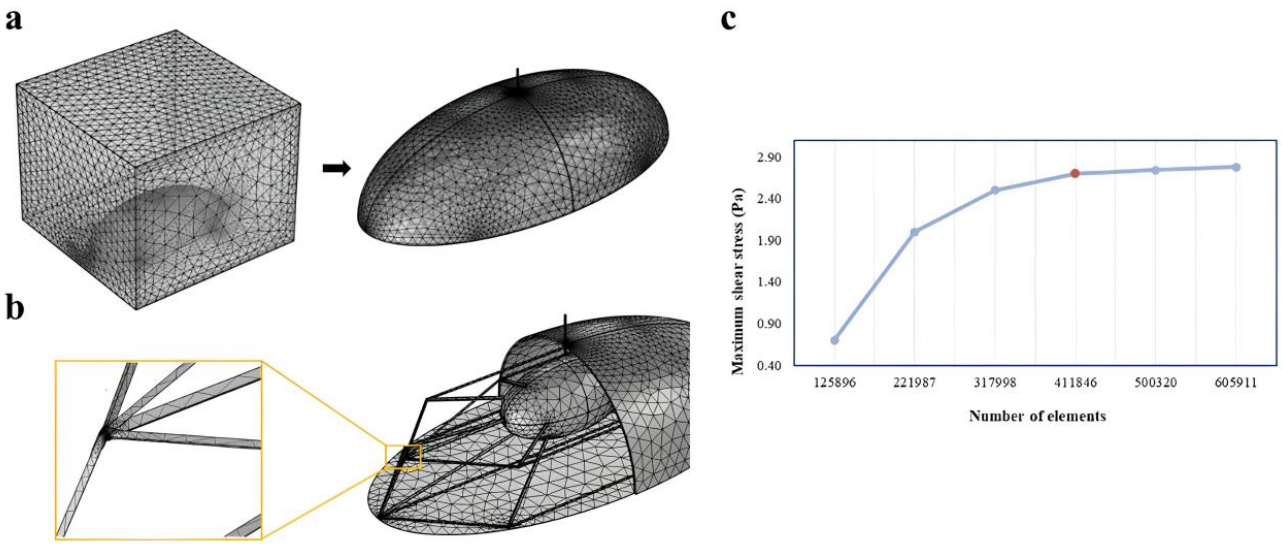

Fig. 3. (a) Computational mesh for bioreactor containing the MSC. (b) Generated mesh for cellular components. (c) Evaluating a cell's maximum von Mises stress while changing the number of elements inside the computational network.

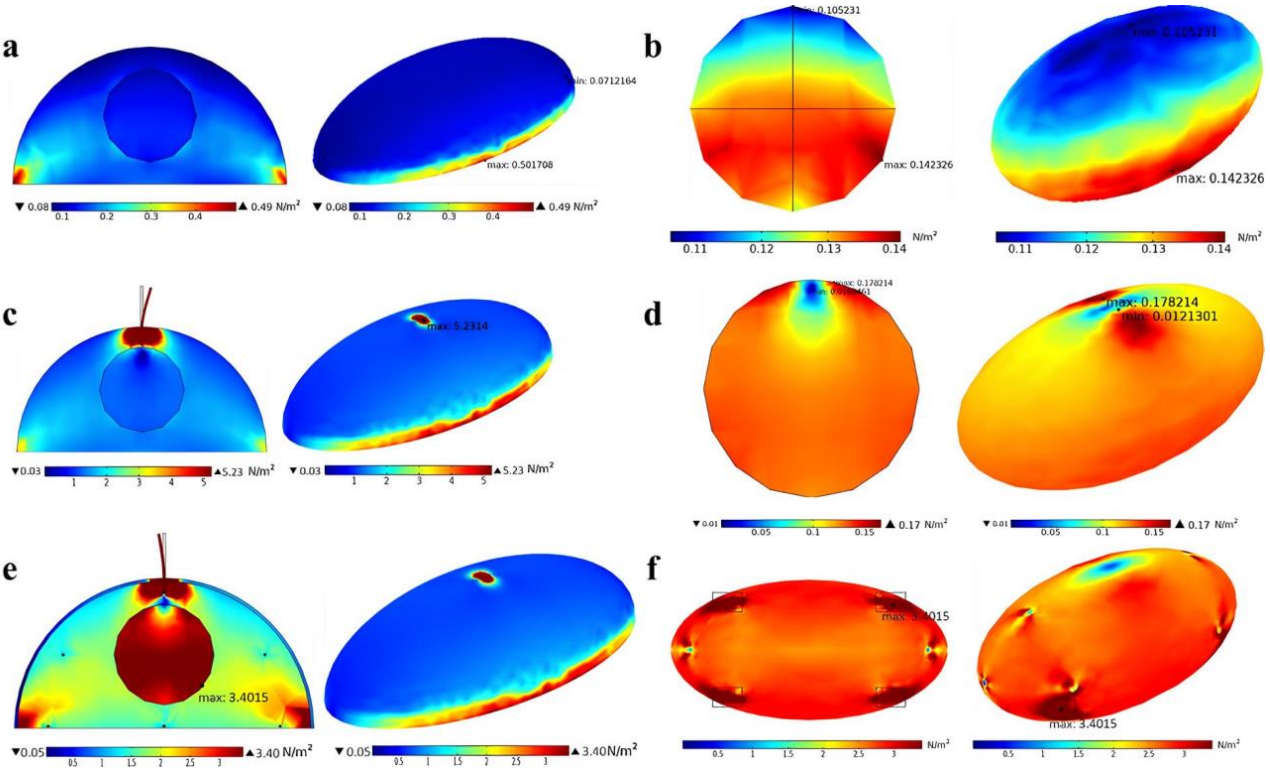

Fig. 4. FSI simulation results in cross-sectional and 3D views in a steady-state condition. (a) Graphical representation of von Mises distribution in the first model, including the cytoplasm and the nucleus. (b) shows the distribution of von Mises stress on the nucleus. (c) represents the von Mises distribution in the second model, including cytoplasm, primary cilium, and the nucleus. (d) shows the distribution of von Mises stress on the nucleus. (e) represents the von Mises distribution in the third model, including cytoplasm, primary cilium, cytoskeleton components, and the nucleus. (f) shows the distribution of von Mises stress on the nucleus.

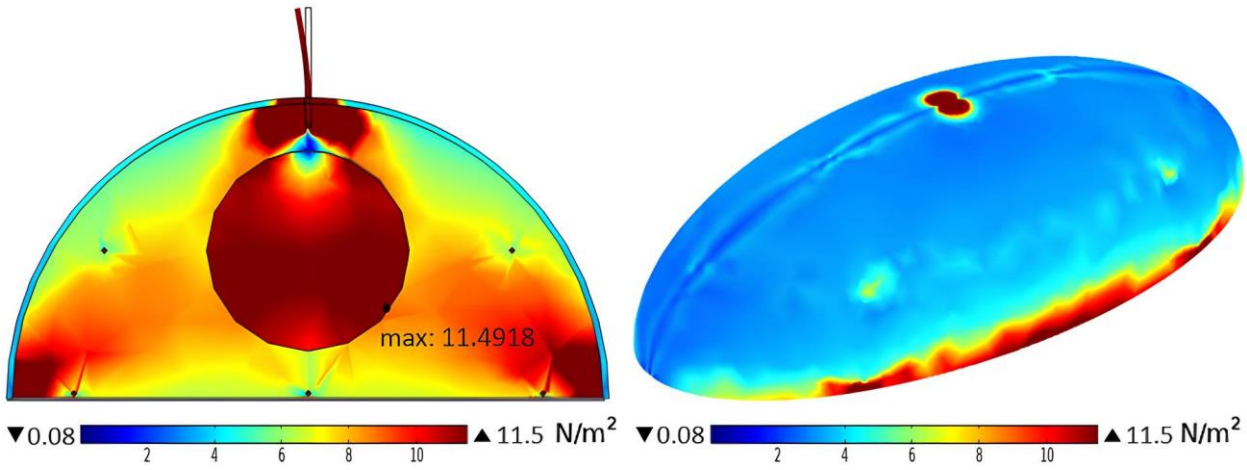

Fig. 5. Graphical representation of von Mises distribution in the fourth model, including cytoplasm, primary cilium, cytoskeleton components, and the nucleus in cross-sectional and 3D views in an oscillatory fluid flow regime.

steady-state conditions. For example, by adding the primary cilium, stress within the cell increased significantly, and by considering the cytoskeleton components, the maximum stress was experienced by the nucleus, which shows how stress can contribute to cellular processes.

3- 2- Oscillatory flow

To investigate the impact of oscillatory flow on all of the designed cellular components, a model was considered including the cytoplasm, nucleus, primary cilium, and cytoskeleton. The maximum magnitude of von Mises stress occurred at the junction of the microtubules and nucleus at t = 0.25 s; however, the value was 11.5 Pa, three times higher than the corresponding value in the steady state condition. By comparing models of steady state regimes (Fig. 4e, f) and oscillatory (Figure 5), it was found that the distribution of von Mises stress on the actin cortex under oscillatory fluid flow was uniform and symmetrical. Moreover, the magnitude of stress at the center of the cell, where actin filaments and microtubules were considered, was four times greater than in the steady state. Figure 6(a) shows the cross-section to analyze the shear stress and the hydrodynamic pressure along the channel. According to Fig. 6b, under oscillatory flow, the average hydrodynamic pressure was much higher than in the other models due to the greater inlet velocity. In all existing models, the values decreased linearly so that it experienced a zero-pascal pressure at the outlet. Figure 6(c), shows the different wall shear stress (WSS) values for all the models, where WSS was greater in the oscillatory regime due to a greater velocity. The differences in WSS and pressure diagrams between the models of the steady state condition, where the cell components were studied separately, were not remarkable, although the diagram in the model in an oscillatory regime, including all the mentioned cell components, it showed great changes.

3- 3- A comparison of von Mises stress in different cellular components.

In this study, we have compared the maximum von Mises stress experienced by different components in three different oscillatory regimes, 0.5, 1, and 2 Hz, at t=0.25 s to evaluate the importance of each component in cellular responses, which are shown in Figure 7. The greatest values occurred in the nucleus and cytoskeleton, and the smallest in the cytoplasm. The higher stress in the nucleus was due to its greater Young's modulus, as it is the stiffest organelle in the cell [14]. The maximum von Mises stress experienced by the nucleus at a frequency of 2 Hz, which was about 12 Pa; however, at 0.5 and 1 Hz the amount of von Mises stress was nearly close to 2 Hz. The stress in the microtubules was similar to that of the nucleus at all frequencies, but nearly three times greater than in the actin filaments. According to the results, the most significant deformation occurred in the bases of the primary cilium, where the mechanism of stress transfer occurs to the cytoskeleton. The primary cilium experienced large deflection under fluid flow stimulation, which resulted in large membrane strains occurring locally around the base of the cilium, as shown in Figures 4 and 5. Therefore, we have reported the effect of cilium length and mechanical properties, which were related to the resulting shear stress and strain. Figure 8, shows a particular relationship between the length of the primary cilium and cell membrane strain, cilium's tip deflection, and maximum shear stress. For a cilium length of 4 µm, which was like that observed *in vitro* 39]], the cilium tip deflection was 0.77 µm. This was a 78% increase for a 10 µm cilium, which was considered to be the longest. Therefore, the longer the cilium, the greater the stress and strain experienced by the cell. This clearly shows that the cilium acts as a booster for mechanical simulations. Also, a rigid cilium with Young's modulus of 1057000 Pa experiences less stress due to a greater elastic modulus, and the tip deflection was 0.37 µm, which was 51% less than the

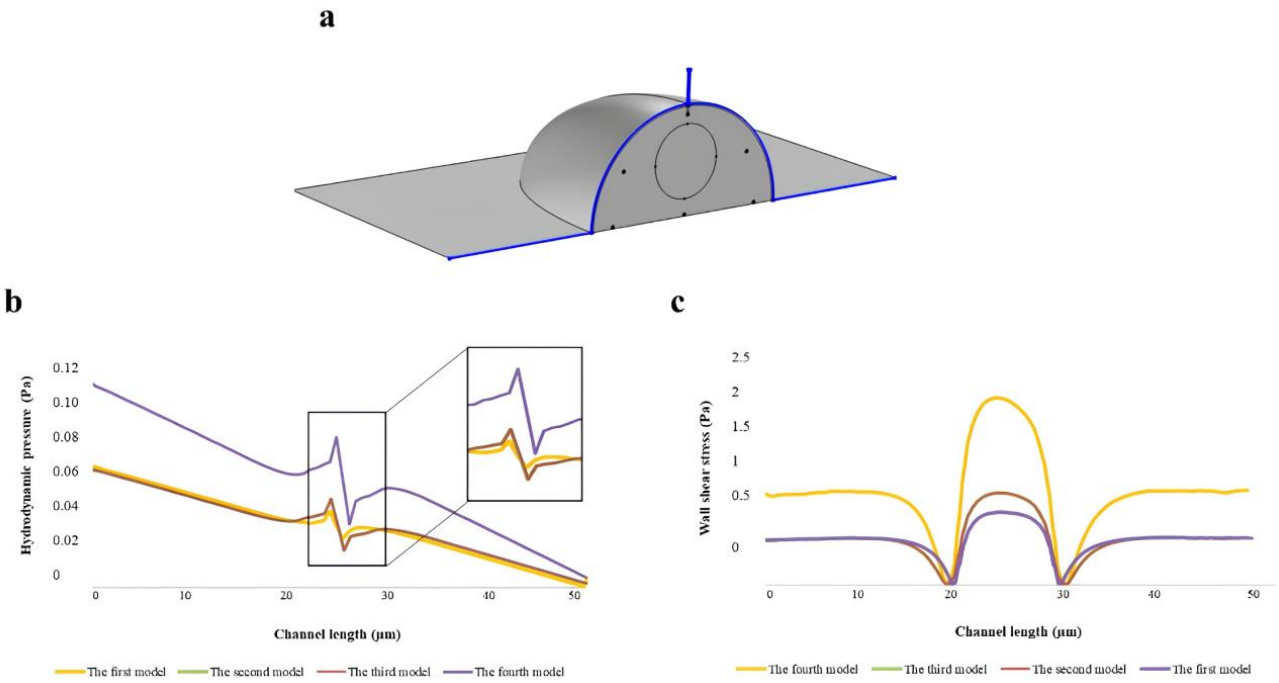

Fig. 6. (a) Cross sectional view of the channel including cell to investigate the shear stress and hydrodynamic pressure along it. (b) The average of hydrodynamic pressure and (c) the wall shear stress.

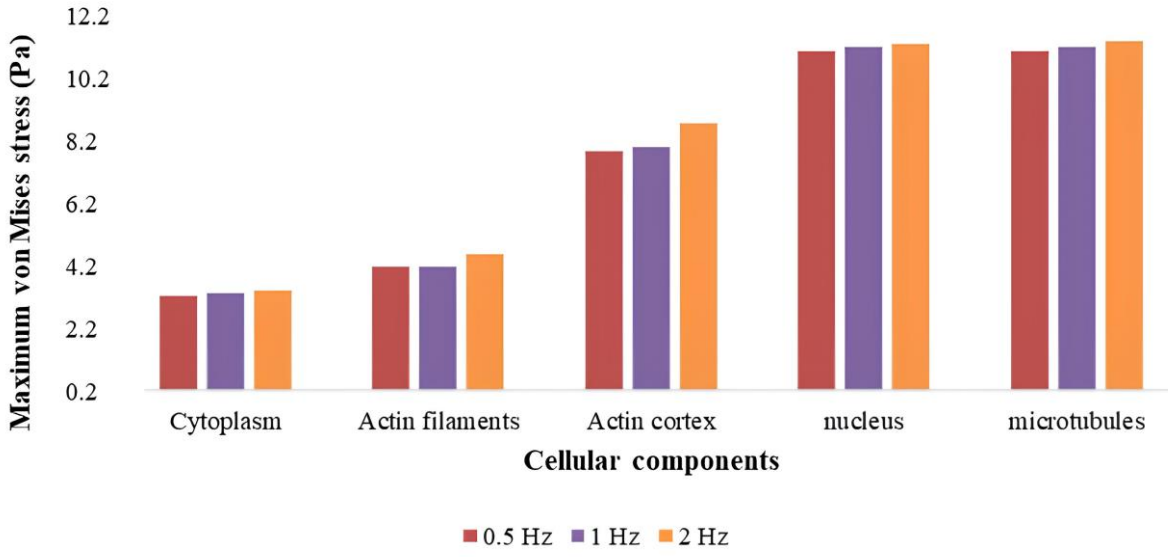

Fig. 7. The effect of different oscillatory flow regimes on the maximum von Mises stress in the cytoplasm, actin filaments, actin cortex, nucleus, and cell microtubules at t=0.25 s.

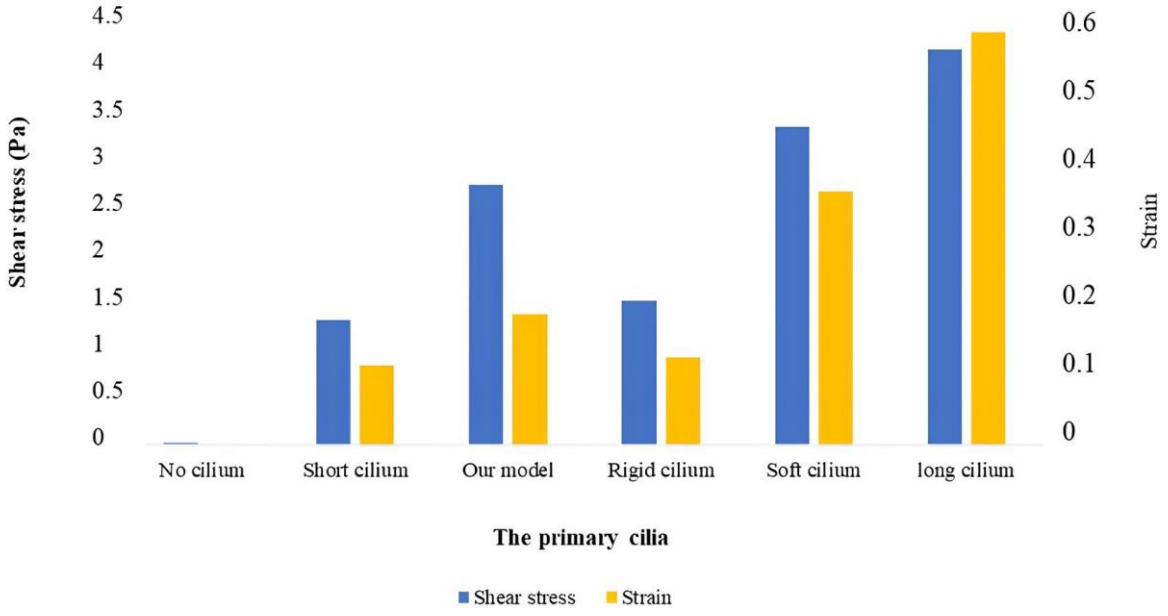

Fig. 8. The maximum shear stress and strain according to changes in length and stiffness of cilia at t=0.25 s in oscillatory fluid flow.

Table 2. The cilium properties in different cell models are compared to the model which has been used in this study, to examine the effects of each one on the maximum shear stress, strain, and cilium's tip deflection.

Model	Young's modulus (Pa)	Length (µm)	Maximum Shear stress (Pa)	Maximum strain	cilium's tip deflection (µm)	Reference
No cilium	-	-	0.018	0.00005	-	-
A short cilium	178000	3	1.3	0.11	0.21	[42]
This study	178000	4	2.7	0.18	0.77	[27]
A rigid cilium	1057000	5	1.5	0.12	0.37	[53]
A soft cilium	17000	5	3.3	0.35	2.3	[53]
A long cilium	178000	10	4.1	0.57	3.61	[53]

real model as shown in Table 2.

4- Discussion

MSCs have a variety of mechanisms to sense and respond to different mechanical stimuli [40]. Mechanical forces transmitted through the cell directly affect nuclear shape and function. Thus, gene expression and numerous processes will occur at the cellular level [41]. The current computational simulation seems to be effective in determining how is the mechanical responses of cells to shear forces and which components of the cytoskeleton affect cellular responses when the external forces are changed. As the perception of the role of cell components under mechanical stimulations is a significant step in investigating the mechanotransduction process, the results were presented in four different models, including oscillatory and steady flow conditions.

In several experimental studies [42, 43], the primary cilium had a critical influence on MSCs commitment and skeletal deformation, which facilitated signaling by enhancing reaction kinetics or bringing specific reaction partners together. By comparing the model including the cilium with the model where it was ignored, the importance of the primary cilium can be clearly understood due to the higher von Mises stress and the maximum stress that occurred

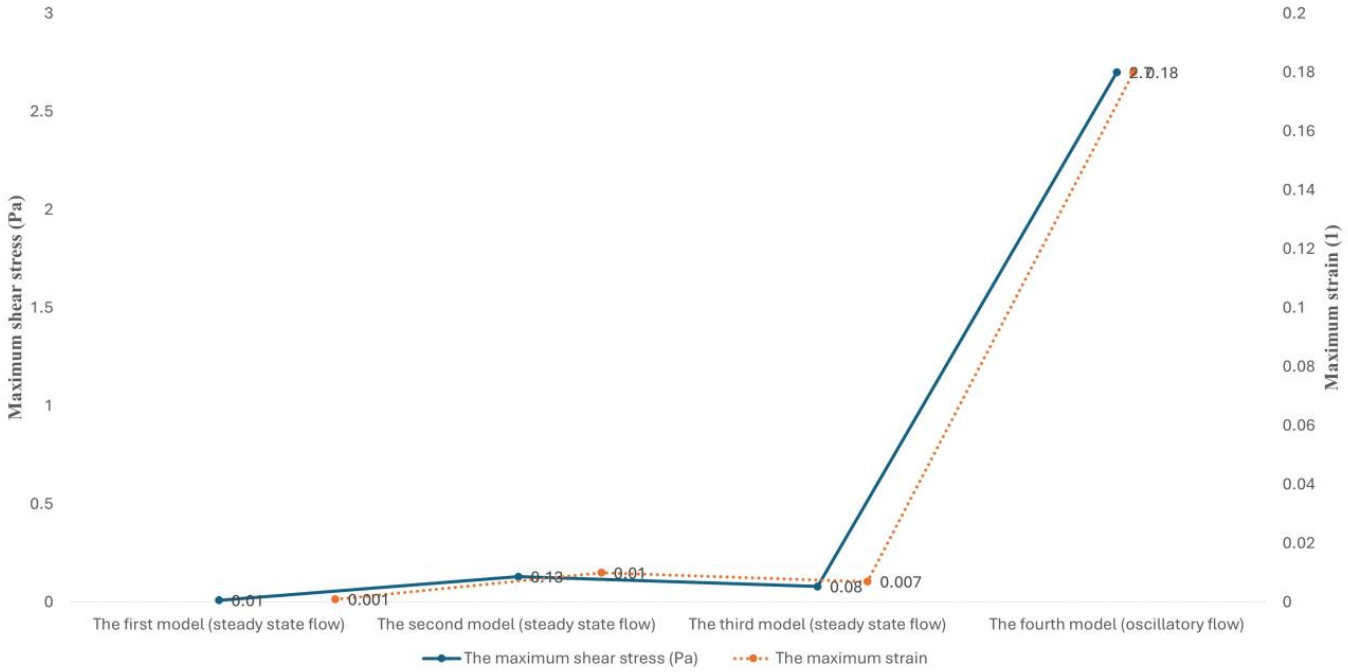

Fig. 9. A comparison between different amounts of the maximum shear stress in both steady and oscillatory flow conditions.

in the transition zone. It is said that the primary cilium's function as a flow sensor in living organisms relies on how it is positioned in the lacunar cavity, particularly its ability to connect to the ECM, which has not been confirmed yet [39]. A former report [13], suggested that actin filaments appear to be essential elements for osteogenesis. This indicates the importance of these organelles in the transduction of the signaling pathways [44]. Thus, according to our results in Figure 4 (e) and (f), in which the cytoskeleton components have been considered, the maximum von Mises stress amount has been increased in the nucleus compared to the model in which they were not considered. The von Mises stress was increased on the nucleus surface, which was about the area of connection between microtubules and the nucleus. As a result, cytoskeleton components resisted high stress and compromised an interconnected mechano-sensor that transduced mechanical signals into biological changes affecting MSC differentiation. Therefore, many studies have laid the groundwork for microtubules as mechanotransducers in bone as fluid shear stress causes the microtubules to be rearranged and their density to be increased [45]. This study also reported the role of microtubules in stress transmission to the nucleus, where stress was almost doubled when microtubules were considered.

Inducing shear stress on MSCs leads to enhanced cell growth and changes in signaling pathways [46]. Generally, the shear stress of $\tau_w > 0.6$ Pa has been implemented to stimulate an osteogenic response in bone cells. Exposure to small amounts of shear stress (0.03–0.27 Pa) can trigger osteogenic differentiation, leading to higher levels of osteopontin and osteocalcin expression [47]. However, [48] conducted a study that indicated a scaffold level WSS between 5 to 15 mPa might enhance mineralization and speed up the osteogenic differentiation of MSCs. The results of the model in an oscillatory regime showed that the maximum von Mises stress and shear stress are at the junction of the nucleus and microtubule filaments, which were 11.5 Pa and 2.7 Pa, respectively. The results we obtained show increased intracellular stress values in response to oscillatory flow, which are supported by the results of multiple experimental studies. However, in a steady-state condition, the amount of shear stress was insufficient to induce an osteogenic effect. Figure 9 shows a comparison between steady state and oscillatory conditions, in which different amounts of the maximum shear stress in each condition are mentioned. It is also important that the biological response of cells has been linked to mechanosensation and mechanotransduction [49]. This means that the measurable biochemical parameters are generally connected to stress and strain levels inside the cells. As a result, thorough empirical assessments are necessary to fully evaluate the condition of oscillatory sheared MSCs, gauge the impact of frequency on downstream regulatory effects, and decide whether low or high frequencies are preferable. For example, the flow regime of 2 Pa and 2 Hz, expected to happen in a living organism, causes the most strong and consistent increase in osteogenic gene expression in vitro [11].

In Figure 6(b), the plots highlight situations where the

cytoskeleton did not have a major effect on the hydrodynamic pressure changes on the wall. In contrast, the presence of primary cilium caused greater pressure compared to the model, which was ignored. On the other hand, in the oscillatory condition, the flow led to a greater hydrodynamic pressure. Furthermore, Figure 6(c), shows that the shear stress experienced by the cell in the model, in which the primary cilium was not considered, was less than its magnitudes in the other models. As a result, the role of the primary cilium on shear stress changes can be understood. According to the results, the advantages of using oscillatory flow due to a greater amount of shear stress are clear. The findings of an experimental study by [46] reveal that MSCs display a temporary elevation in intracellular Ca^{2+} and an increase in cell proliferation rate when exposed to oscillatory fluid flow. Additionally, there is an upregulation in osteoblastic gene expression and a reduction in ALP activity. Three frequencies were considered for comparison; however, the frequency of 1 Hz is the most common one in experimental and computational studies. For instance, [37] studied how MSCs' TRPV4 calcium channels react to shear stress caused by oscillatory fluid flow at 1 Hz, which simulates the expected physiological mechanics in the bone marrow. Their studies revealed that the application of oscillating fluid flow, resulting in a shear stress of 1 Pa, led to a 1.53-fold increase in intracellular calcium levels. The results in Figure 7 were in contradiction with an experimental report [11] since significant changes were reported when the frequency was changed. This can be due to the point that the amount of stress and strain experienced by the cell was not mentioned at different frequencies, and only the biochemical factors were considered as cellular responses.

The role of cilium in modulating osteogenic mechanotransduction pathways, particularly in MSCs, has been an area of interest in recent years [50]. Figure 8 shows the effect of length and Young's modulus on stress and strain in the primary cilium. By comparing different lengths and mechanical properties of the primary cilium, it has been shown that the maximum and minimum shear stress occurred in the long primary and short primary cilium, respectively. So, as the other studies showed [51] in this study, the magnitude of local stimulation was highly dependent on the length of the primary cilium;, longer cilium led to much higher tip deflections compared to the short cilium, which experienced the lowest and resulting membrane strains. Under fluid flow stimulation, our models predicted that long primary cilium undergoes large deflections and induce significant membrane strains at the base, which was qualitatively consistent with previous computational [39] and experimental [52] studies. Also, the stiff cilium experienced less strain than the soft cilium. Furthermore, our results indicate that shear stress experienced by stiff (which was assumed in our model) and short cilium falls within the range of osteogenic differentiation.

This study provides a computational basis for exploring MSCs mechanotransduction. However, there were some limitations, such as the omission of intermediate filaments due to high computational costs. There was a reasonable accordance between the computational and experimental results as mentioned. However, some of the obtained results cannot be thoroughly compared with experimental results due to the need for more information about the amount of stress and strain in cellular components. Measuring the intracellular stress distribution in cells is experimentally challenging due to the inherently multidisciplinary expertise required to conduct and interpret these measurements. In addition, the mechanical properties of living materials can change actively in response to perturbation, causing the tissue to compact more or less under constant force. For future works, a more comprehensive cell model using microscopic imaging, including intermediate filaments and integrins, is valuable to be consider. Additionally, the current cell model could be studied with the presence of a tissue engineering scaffold to provide a more accurate prediction of cell responses.

5- Conclusion

Bone tissue engineering is an increasingly popular alternative to autografts and synthetic implants. However, it is not yet fully understood how mechanosensitive pathways in MSCs are affected by being cultured in a mechanical environment. In this study, an FSI model was developed to characterize the mechanical behavior of individual MSCs within a channel under both steady and oscillatory fluid flow. This work proposed different cell models, including cellular components separately, where the key finding was that the primary cilium influences osteogenic potency, associated with changes in primary cilium length and Young's modulus. The results of this study help to establish whether the primary cilium can function as a mediator *in vivo*. Furthermore, the cytoskeleton and nucleus interacted in a complex way, with one affecting the other, which would have a significant impact on gene expression and differentiation. Therefore, it is vital to consider the effect of oscillatory flow on promoting MSC osteogenesis. However, there was no significant difference in the amount of shear stress among the 0.5 Hz, 1 Hz, and 2 Hz flow regimes. In addition, a greater amount of von Mises stress on the cortex was reported rather than actin filaments. This information is valuable for guiding the search for new experimental methodologies to measure cortex thickness. The current computational study provides new insight into the development of BTE applications and novel platforms for mechanotransduction studies.

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