A fluid–structure interaction model to characterize bone cell stimulation in parallel-plate flow chamber systems

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Bone continuously adapts its internal structure to accommodate the functional demands of its mechanical environment and strain-induced flow of interstitial fluid is believed to be the primary mediator of mechanical stimuli to bone cells \textit{in vivo}. \textit{In vitro} investigations have shown that bone cells produce important biochemical signals in response to fluid flow applied using parallel-plate flow chamber (PPFC) systems. However, the exact mechanical stimulus experienced by the cells within these systems remains unclear. To fully understand this behaviour represents a most challenging multi-physics problem involving the interaction between deformable cellular structures and adjacent fluid flows. In this study, we use a fluid–structure interaction computational approach to investigate the nature of the mechanical stimulus being applied to a single osteoblast cell under fluid flow within a PPFC system. The analysis decouples the contribution of pressure and shear stress on cellular deformation and for the first time highlights that cell strain under flow is dominated by the pressure in the PPFC system rather than the applied shear stress. Furthermore, it was found that strains imparted on the cell membrane were relatively low whereas significant strain amplification occurred at the cell–substrate interface. These results suggest that strain transfer through focal attachments at the base of the cell are the primary mediators of mechanical signals to the cell under flow in a PPFC system. Such information is vital in order to correctly interpret biological responses of bone cells under \textit{in vitro} stimulation and elucidate the mechanisms associated with mechanotransduction \textit{in vivo}.

1. Introduction

Bone is a highly efficient material whose internal tissue structure continuously adapts to accommodate the functional demands of its mechanical environment. It is generally accepted that this process is controlled at a cellular level, whereby mechanosensitive osteocytes regulate remodelling activity by osteoclasts and osteoblasts in response to external mechanical stimuli imposed on the cell. It is thought that this response to external mechanical signals may be caused by either (i) direct stimulation of the cell body as a result of mechanical strain in the extracellular tissue structure or (ii) indirect stimulation of the cell body resulting from strain-induced fluid flow through the lacuno-canaliculal network [1,2]. Given that the necessary mechanical strains required for stimulatory response (typically 1\%) exceed the fracture strain (0.4\%) of the tissue itself, it has been hypothesized that indirect stimulation of the cell body resulting from load-induced fluid flow may be the main mechanism by which bone cells sense their mechanical environment [2–5]. Owing to the practical difficulties in ascertaining whether interstitial fluid flow is indeed the primary mediator of mechanical stimuli in the \textit{in vivo} environment, much of the evidence supporting this theory has been established through \textit{in vitro} investigations that have observed cellular activity in response to fluid flow imposed by perfusion chambers [4,6–10]. It has been shown that bone cells exhibit important biochemical responses, in the form of intracellular calcium...
production [11–13] and the release of signalling molecules such as nitric oxide (NO) and prostaglandin E2, when subjected to various flow regimes [4,7,12,14,15]. While such in vitro experiments have identified key mechanisms involved in the mechanotransduction process, the exact mechanical stimulus being imparted to cells within a monolayer is unknown [16] and it is not clear whether the mechanical stimulation is comparable between different experimental systems or, more importantly, is representative of physiological loading conditions experienced by bone cells in vivo.

Parallel-plate flow chamber (PPFC) systems have been used extensively for in vitro investigations of bone mechanotransduction [4,7,12,14,15]. These systems rely on a pressure gradient to drive the fluid medium through the length of the chamber and may operate under steady-state, pulsatile or oscillating flow regimes [4,6,7,9,10,13,17–19]. To date, the main design criteria associated with PPFCs has been to establish a suitable homogeneous wall shear stress (WSS) profile in the region where cultured cells are located [20]. Computational fluid dynamics (CFD) approaches have been applied to predict the mechanical stresses acting on a single-cell monolayer [21] and multi-cell monolayer arrays [22,23] under laminar flow in PPFC systems. Studies have predicted that there is significant amplification of shear stress (approx. threefold) imparted on the cell membrane owing to the disturbance of the flow in the vicinity of the cell [21–23] and that a large spatial variation of the target WSSs may exist when different flow setups are compared [22]. As such, comparing outcomes of specific cell responses may not be appropriate between different PPFC systems. Furthermore, the appropriateness for such systems in replicating the in vivo loading environment remains unclear, since the applied in vitro mechanical stimulus are not well defined [22]. Experimental observation of osteocytes subject to in vitro fluid flow have shown that different cells within a monolayer may not be receiving the same mechanical stimulus resulting in different levels of intracellular calcium and NO expression within the cell population [12].

The mechanical stress state within PPFC systems is further complicated when the pressure differential required to drive the fluid medium through the channel region is considered. Huesa et al. [16] have recently shown that the operating pressures of some PPFC systems (which may be in excess of 1 kPa) are actually sufficient to stimulate a biochemical response in primary osteoblasts in a purely hydrostatic setting. Importantly, they conclude that PPFC systems that operate by applying a predefined calculated shear stress to the monolayer of cells may produce biochemical responses that are a result of a combined stimulus (i.e. both pressure and shear stress) rather than a response to the shear stress alone. To date, computational studies have focussed on predicting the shear stress under fluid flow but no study has ever considered the possibly significant contribution of the fluid pressure in PPFC systems on cellular deformation under this type of mechanical stimulus.

Previous computational models of bone cells have either used solid mechanics approaches to characterize cellular deformation under shear stress [14] or fluid dynamics modeling, wherein the biological tissues were assumed to be rigid for the purposes of understanding fluid flow and shear stresses [21–23]. In reality, osteoblastic cells are composed of an elastic cell membrane that deforms in response to the external fluid flow imposed by mechanical loading. To fully simulate this behaviour represents the most challenging multi-physics problem, which is too complex to be solved analytically and until recently computational methods were also inadequate. Latest advances in computational resources and software have provided tools that can simulate the interaction between deformable structures and adjacent fluid flows. Such techniques have been used recently to understand the deformation of osteoblast cells under fluid flow using a weakly coupled (one-way) fluid–structure interaction (FSI) scheme [24]. However, cellular deformation can also perturb fluid flow and strongly coupled (two-way) FSI schemes have never been applied to model the complex behaviour of bone cells under mechanical stimuli imposed in vivo and in vitro. To fully understand the cellular mechanisms associated with the mechanotransduction process, it is necessary to characterize the exact nature of the fluid–solid interactions and mechanical stimulus imposed on the cell monolayer within in vitro fluid flow regimes generated by PPFC systems.

In this study, we use a FSI computational approach to investigate the nature of the mechanical stimulus being applied to a single cell under fluid flow within a PPFC system. A multilevel methodology is developed whereby a global CFD model is used to characterize the mechanical shear stresses imposed within a PPFC system, while a local FSI model, informed directly by the predictions of the global model, is used to characterize nature of the deformation experienced by an osteoblast cell subject to steady-state laminar flow. These studies vary both the pressure and WSS profiles within the PPFC system independently, which allows the individual contribution of pressure and WSS on cellular deformation to be studied in isolation of one another. Such information is vital in order to correctly interpret biological responses of bone cells under in vitro mechanical stimulation and elucidate the mechanisms associated with mechanotransduction in the in vivo environment.

2. Material and methods

2.1. Parallel-plate flow chamber system

The main objectives of the current study are (i) to characterize the mechanical stresses acting in the channel region of a PPFC system and (ii) to characterize the resulting cellular deformation arising from fluid–solid interactions at the cell level under the applied fluid flow regimes.

A schematic outlining of the PPFC device under investigation in this computational study is shown in figure 1a. This is an in-house custom-built system, which uses a syringe pump to apply a constant volumetric flow rate at the inlet to drive fluid into an inlet reservoir, through the channel region to the outlet reservoir and finally exiting at the outlet, which is at atmospheric pressure. Within the chamber, cells are plated onto a fibronectin-coated glass coverslip that forms part of the bottom plate in the channel region. The flow system may apply steady state or oscillating flow at a range of volumetric flow rates and frequencies and within the channel region, a calculated fluid shear stress may be applied to the cell population.

For steady, fully developed laminar flow between two infinite parallel plates, the magnitude of the WSS ($\tau_w$) on the bottom plate of the channel may be conveniently expressed in terms of the volumetric flow rate ($Q$) using the following relationship [20,25]:

$$\tau_w = \frac{6Q\mu}{bh^2}. \quad (2.1)$$
where $\mu$ is the fluid viscosity and $b$ and $h$ are the channel width and height, respectively. In general, shear stresses of $\tau_{w} > 0.6$ Pa have been used to stimulate an osteogenic response in bone cells. The current PFPC system has been used to investigate the response of MLO-Y4 mouse osteocyte cells under an oscillatory fluid flow stimulus of approximately $\tau_{w} = 1.2$ Pa peak shear stress (calculated according to equation (2.1)) at a frequency of $1$ Hz [26]. Figure 1b shows that, under this type of mechanical stimulus, there is an increase in the expression of COX-2 compared with samples that were cultured in static conditions. To characterize the exact mechanical stimulus being imparted on the cell monolayer and understand the reason for a biochemical response during such an experimental test, the current study employs a novel multilevel methodology using both CFD and FSI analyses, which are outlined in detail below.

2.2. Global computational fluid dynamics model

In order to quantify the mechanical stresses in the PFPC system, a global CFD model is developed to represent the fluid flow region, as shown in figure 2a. The global CFD model considers fluid flow entering the inlet reservoir, flowing through the channel region to the outlet reservoir, as shown in figure 2a. The global CFD model also considers the tubing which connects the flow chamber to the syringe-pump and atmospheric outlet. This consists of a 200 mm length tubing (not shown) with an inner diameter 3.2 mm connecting at both the inlet and outlets. Owing to the symmetry of the flow device, the global CFD model only considers one half of the fluid flow system to reduce computational time.

The properties of the fluid medium are assumed to be equivalent to water, an incompressible Newtonian fluid having a dynamic viscosity of $\mu = 8.99 \times 10^{-4}$ kg ms$^{-1}$ and density of $\rho = 997$ kg m$^{-3}$. A constant uniform velocity profile was assumed at the inlet, while a no-slip boundary condition is assumed for all chamber walls. A static pressure boundary condition is prescribed at the outlet where a full traction condition was specified. A symmetry condition that assumes zero flux of all quantities across a boundary is enforced along the mid-plane and side boundary of the local FSI model, while a no-slip condition was enforced on the cell surface and on the upper and lower boundaries of the local FSI model. The local FSI model contains a solid deformable domain to represent an osteoblast that is adhered to the bottom plate of the channel region, as shown in figure 2b. This cell is an idealized representation of an osteoblast bone cell whose dimensions are based on experimental observations of osteoblast cells in culture and are given in figure 2b. In this representation of an osteoblast, the nucleus of the cell is assumed to be an ellipsoid with a major diameter of 8 $\mu$m and a minor diameter of 4 $\mu$m. In the local FSI model, it is the cell membrane that acts as the fluid–solid interface, allowing the exchange of data (i.e. forces and displacements) between the fluid and solid domains during the FSI solution phase.

The two-way FSI analysis is carried out using the ANSYS Multiphysics platform through a bi-directional coupling of the ANSYS CFX solver to the ANSYS Structural finite element (FE) solver. The coupled simulation follows a staggered iteration approach, whereby the fluid equations are solved and the resulting fluid stress tensor acting at any fluid–solid interfaces (in this case the cell membrane) is applied as the boundary condition on the solid domain, where resulting deformations are relayed back to the fluid domain and the solution continues through further iterations. For an incompressible Newtonian fluid, the stress tensor acting at the interface boundary between the fluid and solid domains may be characterized by the following expression:

$$\sigma_{ij} = -p\delta_{ij} + \mu \left( \frac{\partial v_i}{\partial x_j} + \frac{\partial v_j}{\partial x_i} \right),$$

where $\delta_{ij}$ is the Kronecker delta, $v_i$ is the velocity of the fluid in the $i$-direction, $p$ is the hydrodynamic pressure and $\mu$ is the dynamic viscosity of the fluid. For an arbitrary point on the

**Figure 1.** (a) Parallel plate flow chamber system under investigation: (i) syringe-driven pump; (ii) inlet tubing; (iii) inlet reservoir; (iv) channel region; (v) outlet reservoir; (vi) outlet tubing; (vii) atmospheric outlet and (b) Cox-2 expression in MLO-Y4 mouse osteocyte cells following 1 h of laminar oscillatory fluid flow (OFF) stimulus (approximately 1.2 Pa peak shear stress at 1 Hz). (Online version in colour.)
boundary of the fluid domain, the forces acting are both normal (because of the hydrodynamic pressure) and tangential (because of shearing effects) and these are transferred to the solid domain using an interpolation algorithm that maps forces between the fluid and solid meshes. The FE method then solves the new boundary value problem in the solid domain and determines the displacement field (i.e. cell deformation) at the fluid–solid interface. A displacement diffusion scheme is adopted to accommodate the deforming fluid mesh and the ANSYS CFX solver calculates the solution for the newly induced fluid flow field. The current solution procedure is calculated for steady-state conditions over one multi-field load step and, although there is no concept of elapsed time within this framework, the ANSYS CFX solver does make use of a false time-step as a means of under-relaxing the equations as they iterate towards a final solution but this is not shared in any way with the

### Table 1. Parameters for local FSI model to examine the effect of volumetric flow rate.

<table>
<thead>
<tr>
<th>flow rate (cm³ min⁻¹)</th>
<th>Reynolds number, Re</th>
<th>WSS (Pa)</th>
<th>P₀ (Pa)</th>
<th>vₑ (mm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>16.786</td>
<td>1</td>
<td>81</td>
<td>37.46</td>
</tr>
<tr>
<td>9</td>
<td>33.57</td>
<td>2</td>
<td>162</td>
<td>74.9</td>
</tr>
<tr>
<td>13.5</td>
<td>50.358</td>
<td>3</td>
<td>243</td>
<td>112.3</td>
</tr>
</tbody>
</table>

### Table 2. Parameters for local FSI model to examine the effect of pressure.

<table>
<thead>
<tr>
<th>flow rate (cm³ min⁻¹)</th>
<th>Reynolds number, Re</th>
<th>WSS (Pa)</th>
<th>P₀ (Pa)</th>
<th>vₑ (mm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5</td>
<td>16.786</td>
<td>1</td>
<td>81</td>
<td>37.46</td>
</tr>
<tr>
<td>4.5</td>
<td>16.786</td>
<td>1</td>
<td>162</td>
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<tr>
<td>4.5</td>
<td>16.786</td>
<td>1</td>
<td>243</td>
<td>37.46</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>81</td>
<td>0</td>
</tr>
</tbody>
</table>
Mechanical application solver. Unlike one-way coupling schemes, the framework employed here uses a staggered iteration approach, whereby the solution iterates between fluid and solid domains until convergence is achieved at the fluid–solid interface. Convergence at the fluid–solid interface occurs when the normalized change of forces at the interface between successive stagger iterations is smaller than the predefined convergence tolerance, in this case 1 per cent [28]. This allows a fully implicit solution of both solid and fluid domains within each multi-field step. The two-way FSI strategy allows a fully coupled interaction of forces, which are transferred from fluid to solid domains, and displacements, which are transferred from solid to fluid domains, and provides a suitable platform to characterize the precise mechanisms involved in bone cell stimulation in PFFC systems.

2.3.1. Structural model

For the solid domain, simulations are carried out using ANSYS Structural FE code assuming a static analysis and within the framework of finite deformation theory. Both the cytoplasm and nucleus regions of the cell model are assumed to behave as homogeneous and isotropic solids whose constitutive behaviour may be described using a compressible Neo-Hookean hyperelastic material model whose strain energy density function may be expressed by the following relation [29]:

\[
W = \frac{\mu_0}{2}(\lambda_1^2 + \lambda_2^2 + \lambda_3^2 - 3) + \frac{\kappa_0}{2}(\lambda_1\lambda_2\lambda_3 - 1)^2,
\]

where \(W\) is the strain energy per unit reference volume, \(\lambda_i\) are the principal stretches, \(\lambda_i\) are the deviatoric principal stretches and \(\mu_0\) and \(\kappa_0\) are the initial shear and bulk moduli, respectively. The initial shear and bulk moduli may be related to elastic modulus \(E\) and Poisson’s ratio \(\nu\) using standard isotropic linear elastic relations where \(\mu_0 = E/2(1+\nu)\) and \(\kappa_0 = E/3(1-2\nu)\). For the cytoplasm, an elastic modulus of 4.47 kPa [30] and a Poisson’s ratio of \(\nu = 0.4\) were chosen. The assumption of compressible cell behaviour was based on several experimental studies that have characterized Poisson’s ratio in both osteoblast [31] and chondrocyte [32–34] cell types and determined to be approximately \(\nu = 0.4\). Similar values have been used in a number of previous computational investigations examining the mechanical behaviour of osteoblast cells [14,35]. Meanwhile, it was assumed that the nucleus stiffness was four times that of the cytoplasm region, resulting in an elastic modulus \((E)\) of 17.88 kPa, while maintaining the equivalent Poisson’s ratio \((\nu = 0.4)\). The model was discretized using approximately 30,000 eight-noded hexahedral elements. We assume a homogeneous bond along the entire base of the cell and the fluid flow chamber surface, thus preventing fluid flow beneath the cell body.

2.3.2. Fluid model

The fluid properties and boundary conditions of the local FSI model were the same as those outlined above for the global CFD model. The fluid domain of the local FSI model was discretized with 3 million tetrahedral elements where a significant local refinement was carried out in the vicinity of the solid domain to allow for the smooth resolution of pressure and WSS profiles.

2.4. Parameter variation of flow characteristics

The current study investigates the contribution of hydrodynamic pressure and WSS on cellular deformation within a PFFC system for a range of fluid flow regimes. To characterize the mechanical stimulus exerted on an osteoblast cell, we carry out a range of parameter studies in the global CFD and local FSI models that vary both the pressure and WSS profiles acting within the flow chamber.

2.4.1. Global computational fluid dynamics model

In the global CFD model, we apply a range of volumetric flow rates and characterize the resulting pressure and WSS profiles acting in the channel region. The volumetric flow rates considered are 4.5, 9, 13.5 cm\(^3\) min\(^{-1}\) so that WSSs of 1, 2 and 3 Pa are generated according to equation (2.1). This range of WSSs is typical of those applied to cultured cells using PFFC systems and have been found to be of sufficient magnitude to stimulate a biochemical response [4,6–10] for these types of cells and in our own experiments (figure 1b).

2.4.2. Local fluid–structure interaction model

The results of each case considered in the global CFD model are used to inform the boundary conditions to be applied to the local FSI model. For each of the volumetric flow rates applied to the global CFD model, the pressures and WSSs acting in the centre of the channel (i.e. where the cells are plated) are determined. The volumetric flow rates and pressure conditions are applied to the local FSI model allowing it to emulate the flow regime in the global CFD model. The volumetric flow rates examined in the local FSI model are equivalent to those considered in the global CFD model and for clarity these are referred to in the global context for the entire system (i.e. 4.5, 9 and 13.5 cm\(^3\) min\(^{-1}\)) for all results presented hereafter. Table 1 summarizes the Reynolds’s number, prescribed outlet pressure \((P_o)\), mean velocities in the channel \((v_o)\) and the calculated WSS (determined using equation (2.1)) in the local FSI model for each of the volumetric flow rates considered. It is important to note that in this first parameter study, we only vary the volumetric flow rate but the pressure conditions within the system varies as a consequence, as shown in table 1.

Increasing the flow rate in either the global CFD model or local FSI model affects both the magnitude of pressure and WSS acting in the chamber and in order to decouple the effects of the pressure and WSS, these parameters must be varied independently of one another. Thus, in the second parameter variation study, we vary the pressure acting in the chamber independently of the WSS. This is carried out by applying the same volumetric flow rate to the local FSI model but varying the outlet pressure, which has no effect on the WSS imparted in the channel region. Table 2 summarizes the conditions in the local FSI model for each of the outlet pressure \((P_o)\) cases considered. Also, we examine the effect of applying a pressure in isolation of any WSS by considering a model that applies an

<table>
<thead>
<tr>
<th>flow rate (cm(^3) min(^{-1}))</th>
<th>Reynolds number, (Re)</th>
<th>(h) ((\mu)m)</th>
<th>WSS (Pa)</th>
<th>(P_o) (Pa)</th>
<th>(v_o) (mm s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56</td>
<td>2.098</td>
<td>100</td>
<td>0.5</td>
<td>81</td>
<td>9.3</td>
</tr>
<tr>
<td>4.5</td>
<td>16,786</td>
<td>200</td>
<td>1</td>
<td>81</td>
<td>37.46</td>
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<td>35.95</td>
<td>134.28</td>
<td>400</td>
<td>2</td>
<td>81</td>
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</tr>
<tr>
<td>4.5</td>
<td>16,786</td>
<td>200</td>
<td>1</td>
<td>-10.3</td>
<td>37.46</td>
</tr>
</tbody>
</table>

Table 3. Parameters for local FSI model to examine the effect of WSS.
outlet pressure of $P_o = 81$ Pa, however, there is zero flow at the inlet resulting in $\tau_w = 0$ Pa in the channel region.

Finally, the third parameter study varies the WSS independently of the pressure in the channel region. From equation (2.1), the WSS is a function of the chamber height ($h$) and by varying the value of $h$ in the local FSI model, a number of flow regimes that have different WSSs but have identical pressure profiles may be analysed. Three separate chamber heights are considered thus resulting in three separate WSS values but maintaining a constant outlet pressure for each case. Also, we examine the effect of applying a WSS in isolation of any pressure. This is implemented by for the 4.5 cm$^3$ min$^{-1}$ flow rate case and applying a negative pressure at the outlet, such that in the centre of the channel resulting in a negative pressure at the outlet, such that in the centre of the inlet resulting in

$$w = \frac{\tau_w}{\mu}$$

(2.1), the WSS is a function of the chamber height ($h$) and by varying the value of $h$ in the local FSI model, a number of flow regimes that have different WSSs but have identical pressure profiles may be analysed. Three separate chamber heights are considered thus resulting in three separate WSS values but maintaining a constant outlet pressure for each case. Also, we examine the effect of applying a WSS in isolation of any pressure. This is implemented by for the 4.5 cm$^3$ min$^{-1}$ flow rate case and applying a negative pressure at the outlet, such that in the centre of the channel where the cell is located, the pressure acting is close to zero. The full details of boundary conditions for the third parameter study are summarized in table 3.

### 3. Results

#### 3.1. Global computational fluid dynamics model

The global CFD model was used to characterize the WSS and pressure profiles acting in the channel region of the PPFC system for a number of volumetric flow rates. Figure 3a,b shows the respective WSS and pressure profiles acting along the length of the chamber for each of the flow rates considered, i.e. 4.5, 9 and 13.5 cm$^3$ min$^{-1}$. Figure 3a shows that, once the flow has become fully developed, there is a constant value of WSS acting along the channel for each flow rate considered. With the exception of entrance and exit effects in the channel, the magnitude of this WSS is close to the analytical solution determined using equation (2.1) (i.e. WSS of 1, 2 and 3 Pa). The WSS does not match the analytical solution determined using equation (2.1) exactly because of the finite dimensions of the side walls, which is similar to predictions by Chung et al. [20] who showed that as the width-to-height ratio of the chamber increases, the WSS approaches the analytical solution described by equation (2.1). Figure 3b shows that, as the volumetric flow rate of the system increases, the pressure in the system increases accordingly. This pressure term decreases linearly along the channel length for each volumetric flow rate considered. Figure 3a,b shows the location of the local FSI model with respect to the global CFD model (i.e. the shaded region) and it is the results at these locations of the global CFD model that are used to inform the boundary conditions for the local FSI model. In particular, the pressure acting at the outlet of the local CFD model is of interest and this condition may be determined from the pressure profile in figure 3b (i.e. at X-distance = 10 mm). The velocity profiles in the channel region at X-distance = 8 mm are also shown in figure 3c for each of the flow rates considered. These velocity profiles are fully developed and are used as the inlet boundary conditions in the local FSI model.

#### 3.2. Local fluid–structure interaction model

##### 3.2.1. Effect of flow rate

In the first study, the volumetric flow rate of the system is varied to determine its effect on the cellular strain. The magnitudes of the pressures and WSSs determined from the global CFD model, which inform the boundary conditions of the local FSI model, are summarized in table 1. Figure 4a,b shows the respective WSS and pressure profiles acting along the length of the local FSI model for each of the flow rates considered. With the exception of disturbances

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**Figure 3.** Flow characteristics of the global CFD model: (a) WSS profile (b) pressure profile along the X-direction and (c) velocity profile in the Y-direction.
in the vicinity of the cell, the WSS and pressure profiles shown here for the local FSI model are almost identical to those from the global CFD model in the same region (see figure 3a,b). Figure 4a shows that the WSS is not constant in the vicinity of the cell (i.e. where X-distance = 1 mm) owing to the disturbance in the flow field from the protruding cell in this region leading to significant amplification (almost threefold) of the WSS acting on the membrane of the cell. Figure 4b shows that the WSS is not constant in the vicinity of the cell, the WSS and pressure profiles acting along the length of the local FSI model. In figure 5a, the WSS profiles are superimposed on top of one another, while the pressure acting in the vicinity of the cell is noticeably different for each pressure-outlet condition considered. The effect of pressure on cellular deformation is shown in figure 5c–e, where increasing magnitudes of pressure result in greater equivalent elastic strains within the cell, despite the fact that the applied WSS is identical for all conditions. Interestingly, the magnitude and distribution of equivalent elastic strain in the osteoblast cell (figure 5c–e) for each pressure condition is similar to the previous parameter variation study on the effect of volumetric flow rate (figure 4c–e), suggesting that the contribution of shear stress on cellular deformation in this type of flow system is relatively small.

3.2.3. Effect of wall shear stress
To fully characterize the nature of cell deformation within these types of flow systems, a final parameter study is carried out that considers a variation of WSS independently of the pressure within the system. In this case, the pressure at the outlet was held constant at $P_o = 81$ Pa while three separate WSSs are considered. As the pressure along the length of the channel is dependent upon the volumetric flow rate (figure 4a), to vary the WSS independently of the pressure, the height of the channel was varied while the flow rate was held constant at 4.5 cm$^3$ min$^{-1}$. Three WSSs of $\tau_{w_1} = 0.5$ Pa, $\tau_{w_2} = 1$ Pa and $\tau_{w_3} = 2$ Pa are considered and the resulting WSS profiles for each case considered is shown in figure 6a. Figure 6b shows that for each WSS considered, the pressure profiles in each flow regime are very similar. The effect of the WSS on cellular deformation is shown in figure 6c–e, and indicates that increasing the applied WSS...
has little or no effect on the equivalent elastic strain observed in the osteoblast cell. This is in contrast to the large effect that the applied pressure had on cellular deformation (figure 5c–e) and suggests that within these types of flow systems, it is the pressure term that dominates the mechanical stimulus and the effect of WSS is relatively small.

3.2.4. Isolated pressure and wall shear stress
Finally, the effect of pressure and WSS on cellular deformation are examined in isolation of one another. Figure 7a shows that for the isolated WSS case, a WSS of \( \tau_w = 1 \) Pa is imparted on the bottom wall of the channel, while the pressure at the outlet was chosen such that the pressure

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**Figure 5.** Effect of outlet pressure in the local FSI model: (a) WSS profile and (b) pressure profile along the X-direction. Also shown is equivalent elastic strain in osteoblast cell from FSI simulations for each outlet pressure considered: (c) 81 Pa pressure outlet; (d) 162 Pa pressure outlet and (e) 243 Pa pressure outlet. (Note: the direction of fluid flow in these contour plots is from left to right.) (Online version in colour.)

**Figure 6.** Effect of WSS in the local FSI model: (a) WSS profile and (b) pressure profile along the X-direction. Also shown is equivalent elastic strain in osteoblast cell from FSI simulations for each outlet pressure considered: (c) 0.5 Pa WSS; (d) 1 Pa WSS and (e) 2 Pa WSS. (Note: the direction of fluid flow is left to right.) (Online version in colour.)
acting in the vicinity of the cell is close to zero, as shown in figure 7b. Meanwhile, for the isolated pressure case, a zero-volumetric flux is applied at the inlet, such that the WSS is zero along the channel, see figure 7a, while there is a hydrostatic pressure of 81 Pa acting throughout the channel, as shown in figure 7b. Figure 7c, d shows that the isolated pressure case induces much greater deformation on the cell body than the isolated WSS case. For the isolated pressure case, shown in figure 7c, the magnitude and distribution of strain is similar to that previously observed in figure 6c, e. For the isolated WSS case, the magnitude of the strain is much less than the cases previously examined at this level of WSS ($\tau_{\text{w}} = 1$ Pa), see figure 4c, because of the absence of the pressure term. Also, the strain distribution is somewhat different owing to the fact that loading on the cell is now shear dominated.

4. Discussion

In this paper, a multilevel CFD and FSI analysis has been outlined that characterizes the mechanical stresses in a PPFC system and predicts the resulting deformation in an idealized osteoblast cell subject to a range of laminar fluid flow regimes. The analysis decouples the contribution of pressure and WSS on cell deformation and for the first time highlights that cell deformation in PPFC systems is dominated by the pressure in the channel region not the WSS. This has important implications for understanding in vitro mechanobiology experiments, as the main design criteria for in vitro PPFC systems has been concerned with imparting a predefined WSS to the monolayer of cells and does not consider the pressure in the system. Specifically our results indicate that different PPFC systems may be imparting significantly different mechanical stimuli to cells depending on the design of the chamber (i.e. channel dimensions and the inlet/outlet conditions), which dictates the pressure in the channel. Importantly, this multilevel strategy provides a novel computational method of investigating the cellular deformations occurring during in vitro mechanobiology studies and thus will facilitate cellular mechanisms associated with mechanotransduction to be further understood.

Potential limitations of the current study are mainly concerned with the local FSI model, in particular the representation of a single osteoblast cell as an idealized geometry that does not consider changes in cell morphology because of fluid flow or time-dependent constitutive behaviour. This model only considers a single idealized osteoblast cell subject to a fluid flow stimulus, which could be representative of the beginning of experiments (i.e. after cell seeding and attachment) when osteoblasts are distant from each other, but does not represent the case when cells have proliferated to form a confluent monolayer. Future studies incorporating cell geometries using confocal microscopy imaging could be implemented using the approach we have developed in this study. These studies will require substantial local mesh refinement to resolve all geometric features [36] and may be computationally challenging, particularly in the fluid domain where a very refined mesh is required to resolve pressures and velocities at the boundary layer. Furthermore, the morphology of the osteoblast cell is also assumed to remain unchanged during the analysis which represents a simplification of actual cell behaviour as it has been shown that osteoblast cells tend to elongate and realign when subject to a fluid flow stimulus [37] owing to the rearrangement of the internal actin cytoskeleton. However, cell reorganization is known to initiate following 15 min of stimulation [37], whereas biochemical responses such as NO production have been shown to increase after just 5 min of fluid flow stimulation [17], suggesting that morphological changes should not affect the initial cell response greatly. Finally, there is much

Figure 7. Isolated pressure and WSS cases in the local FSI model: (a) WSS profile and (b) pressure profile along the X-direction. Also shown is equivalent elastic strain in osteoblast cell from FSI simulations for (c) isolated pressure case and (d) isolated WSS case. (Note: the direction of fluid flow is left to right.) (Online version in colour.)
contention in the literature regarding the degree of compressibility of cells and a number of investigations have actually assumed that cells are incompressible \((v = 0.5)\) [36]. Our assumption of compressible behaviour (where \(v = 0.4\)) is based on the properties determined from several experimental investigations [32–34] on similar cell types and the fact that similar values have been assumed in several computational investigations examining cell deformation [14,35,39]. In particular, McGarry [39] investigated the role of compressibility and Poisson’s ratio in chondrocyte cells and determined that assuming incompressible behaviour \((v = 0.5)\) lead to considerable lateral bulging of the cell under parallel-plate compression. This was not consistent with experimental images of compressed cells obtained by Caille et al. [38] and it was found that a compressible cell with a Poisson’s ratio of \(v = 0.4\) provided a better prediction of experimental deformation. Furthermore, Ronan et al. [40] showed that resistance to compressive deformation could be attributed to lengthening of contractile stress fibres in the hoop direction. This is relevant as it should be noted that cells are heterogeneous structures and the overall compressibility is a function of the many components that comprise a cell, including the actin cytoskeleton, and are therefore likely to exhibit a certain degree of compressibility.

The key findings of this study have highlighted the dominant nature of the pressure term on the deformation on an osteoblast cell under a fluid flow stimulus. In previous computational studies of PPFC systems, it is the shear stress that is considered to dominate the applied mechanical stimulus. A number of CFD simulations have highlighted amplifications in the target WSS along the cell membrane [21–23], similar to that observed in our local FSI model (figure 4e), however do not consider the potentially significant contribution of pressure acting on the cell membrane. If cellular response under fluid flow is assumed to be a strain-activated mechanism, then the local FSI model results herewith are in agreement with the experimental studies of Huesa et al. [16] who predicted that the stimulation of cells in a PPFC was probably a result of a combined stimulus of pressure and WSS. Our study shows that increasing the volumetric flow rate of the system led to an increase in the magnitude of the WSS in the channel region and also an increase in the equivalent elastic strain imparted on the osteoblast cell (figures 3 and 4). While there is a direct relation between volumetric flow rate and WSS, as shown by equation (2.1), this design criterion does not consider how a change in volumetric flow rate will affect the pressure in the PPFC system. Previous experimental investigations have shown that increasing the applied shear stress (achieved by increasing the volumetric flow rate) resulted in an increase in the biochemical response of the particular cell under investigation (e.g. an increase in intracellular calcium [12,13] or NO [18] production). The results from our study suggest that the increase in biochemical response observed in these experimental investigations [12,13,18] may have been affected by pressure changes in the fluid flow system (figure 3b) that occur when the volumetric flow rate is varied. Furthermore, the pressure in PPFC systems depends upon a number of other factors, not just the volumetric flow rate, e.g. the dimensions of the channel region and the inlet/outlet conditions of the chamber, and the findings presented here imply that the mechanical stimulus imparted on cells by different PPFC systems and resulting cellular deformation could vary greatly,

even if the target shear stresses were similar, as shown by the results in figure 5. Interestingly, if we apply a theoretical solution for flow in a narrow channel with zero pressure at the downstream end, it may be shown that the ratio of the pressure \((p)\) in the centre of the channel to the WSS \((\tau_w)\) is approximately equal to ratio between the length \((L)\) and height \((h)\) of the channel [41] (i.e. \(p/\tau_w \approx L/h\)). As \(h \ll L\) for most PPFC systems, the pressure term is likely to dominate the mechanical stimulus applied to the cell monolayer. Given that the length and height of the channel region in different PPFC systems can vary significantly, these results suggest that caution is necessary when comparing the biological results of studies conducted using different PPFC systems as the mechanical stimulus being applied directly to the cell may be dissimilar and highlight that future studies using these types of PPFC systems should report the operating pressures of such systems.

However, such theoretical solutions are unable to predict the precise deformation that occurs on the bone cell membrane and the interaction with the adjacent fluid flow and our model predicts that the large normal forces acting on the cell membrane result in compressive deformation on the osteoblast cell body. Under this type of loading, the strain on the cell membrane is relatively low and much of the strain is concentrated at the base of the cell, i.e. where it is adhered to fibronectin substrate, which is clearly evident in figure 4e. This is in contrast to the findings of a FE study by McGarry et al. [14] who applied a shear force to the cell membrane and found greater deformation in the membrane and cytoskeleton regions of the cell than at the cell–substrate interface. The results from our study show how the combined stimulus of pressure and shear stress, which, as is clear from our studies, is representative of \textit{in vitro} fluid flow stimulation, results in a different mode of cell deformation than just a shear force alone. Also, the upper limit of the equivalent elastic strain at the cell–substrate interface is greater than 10,000 \(\mu\varepsilon\) for many of the models presented, which is above the threshold value required to stimulate a biochemical response under directly applied mechanical strain [42]. Interestingly, it has been shown experimentally that focal adhesions at the cell–substrate interface [43] play an important role in the mechanotransduction process under a fluid flow stimulus, as suppressing integrin attachments has been shown to inhibit PGE2 and COX-2 expression [44]. Our results would suggest that, under a fluid flow stimulus, strain concentrations (figure 4e) at the cell–substrate focal adhesions contribute to these specific biochemical responses (e.g. PGE2 and COX-2 expression) more than the deformation of the mechanoreceptors of the cell membrane. Such responses might compliment biochemical expression mediated by primary cilia bending under fluid flow, as has been shown in recent \textit{in vitro} studies [45]. However, whether membrane deformation is sufficient to stimulate stretch-activated ion channels [13] requires further investigation. The computational framework outlined here provides a suitable platform to further examine the effect of fluid flow on both of these phenomena and will be the subject of future investigations.

The PPFC system under investigation in the current study showed relatively low pressures (81–243 Pa) acting in the chamber region when compared with other PPFC devices [10,16], which can have inlet pressures in the kilopascal range, and this magnitude of pressure still showed a dominating effect on cellular deformation, as shown in figures 4
and 5. However, the results from the current study do not intend to discount fluid flow experiments using PPFC systems as these have provided crucial insight into cellular mechanisms associated with mechanotransduction. These results also have implications for other cell types, such as endothelial and chondrocytes, whose biological function is controlled by mechanical stimuli and whose mechanotransduction mechanisms have been tested using PPFC systems [20,46,47]. Importantly, characterizing precisely the mechanical stress state present during these in vitro experiments should provide better insight into the mechanisms associated with cell mechanotransduction and could allow investigators to manipulate in vitro fluid flow regimes (i.e. both pressure and shear components) to more accurately emulate in vivo flow conditions.

5. Conclusions

In this study, we develop a novel multilevel CFD and FSI model to characterize the mechanical stimulation of an osteoblast cell within a PPFC system under a range of fluid flow regimes. By varying the pressure and WSS independently of one another, the analysis has shown that it is the pressure within a PPFC system that dominates the deformation in the cell body. It was found that the contribution of the WSS on cellular deformation was relatively small, which has important implications as this has been the main design criteria for PPFC systems to date. The results also show that the mechanical stimulus being applied by different PPFC systems are not equal as the pressure within these systems are known to vary considerably, even if the target shear stresses are similar. It is therefore important to consider the effects of both pressure and WSS when designing new PPFC systems given that the cell monolayer is likely to receive a combined stimulus from the fluid flow field. Furthermore, it was found that significant strain amplification occurred at the cell–substrate interface, while strains imparted on the cell membrane were relatively low under this type of mechanical stimulus, suggesting that strain transfer through focal attachments at the base of the cell are the primary mediators of mechanical signals to the cell.

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