Measuring the density and viscosity of culture media for optimized computational fluid dynamics analysis of *in vitro* devices

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Abstract

Culture medium is frequently modelled as water in computational fluid dynamics (CFD) analysis of *in vitro* culture systems involving flow, such as bioreactors and organ-on-chips. However, culture medium can be expected to have different properties to water due to its higher solute content. Furthermore, cellular activities such as metabolism and secretion of ECM proteins alter the composition of culture medium and therefore its properties during culture. As these properties directly determine the hydromechanical stimuli exerted on cells *in vitro*, these, along with any changes during culture must be known for CFD model accuracy and meaningful interpretation of cellular responses. In this study, the density and dynamic viscosity of DMEM and RPMI-1640 media supplemented with typical concentrations of foetal bovine serum (0, 5, 10 and 20% v/v) were measured to serve as a reference for computational design analysis. Changes in these properties during culture was investigated with H460 and HN6 cell lines. The density and dynamic viscosity of the media increased proportional to the % volume of added foetal bovine serum (FBS). Importantly, the viscosity of 5% FBS-supplemented RPMI-1640 was shown to increase significantly after 3 days of culture of NCI-H460 and HN6 cell lines, with distinct differences between magnitude of change for each cell line. Finally, measured values were applied in CFD analysis of a simple microfluidic device, which demonstrated clear differences in maximum wall shear stress and pressure between fluid models. Overall, these results highlight the importance of characterizing model-specific properties for CFD design analysis of cell culture systems.

Keywords: culture media, rheology, density, dynamic viscosity, tissue engineering, fluid properties, computational fluid dynamics

1. Introduction

Liquid culture media have been integral to the culture of mammalian cells since the inception of *in vitro* techniques and are composed of a mix of water, essential nutrients, vitamins and factors that support and regulate the growth of cells [1]. Other than the substrate, the culture medium is the other immediate environment that cells contact *in vitro*. In culture devices that involve flow such as bioreactors [2-6] and microfluidic organ-on-chips [7-10], culture fluid is the primary material through which mechanical loading is exerted on cells. However, significantly less attention has been paid to the physical properties of culture medium and how these may affect flow mechanics within these systems compared to considerations such as substrate geometry, tubing dimensions and flow rate [11]. Given the known sensitivity of cells to shear stimuli and particularly where the goal is to study the effects of physiological or pathological shear [12-16], it is essential to characterize precise flow properties and hydrodynamic regimes in order to (i) deliver known and controlled mechanical stimuli to cells, and (ii) correlate these to cellular responses.

Such analysis can be carried out via computational fluid dynamics (CFD) modelling, an effective method for quantitatively determining fluid flow phenomena that may be too complex

or challenging to measure across a whole system or on micron (cell-relevant) scales by experimental means, e.g. by particle imaging velocimetry (PIV) [17, 18], laser doppler velocimetry [19-21], physical probes or sensors. CFD simulations are routinely conducted as part of the design process of fluid systems in engineering and have been increasingly implemented by tissue engineers and the microfluidics community to evaluate flow characteristics within device designs prior to prototyping [22-27]. With appropriate model set up, CFD analysis can provide accurate approximations of flow fields, velocity and stress profiles within tissue culture systems for design refinement [28, 29] and enable more direct study of the biological effects of fluid shear on cells.

The basic principle of CFD modelling is to resolve a set of governing equations that mathematically describe flow behaviours and properties across the discretised geometry (mesh) of a fluid system. The Navier-Stokes and continuity equations (Equations 1 and 2) are the most applicable to modelling incompressible, Newtonian, water-like fluids such as culture medium. These equations are based on conservation of momentum and mass respectively and together, describe the space-time evolution of a velocity field. The equations are as follows:

$$\rho\left(\frac{\delta u}{\delta t} + u.\nabla.\boldsymbol{u}\right) = -\nabla \mathrm{p} + \mu\nabla^2 + f \tag{1}$$

$$\nabla.\boldsymbol{u} = 0 \tag{2}$$

where $\boldsymbol{u}$ ($m.s^{-1}$) denotes the fluid velocity vector, ρ ($kg.m^{-3}$) is the density and μ (Pa.s) is the dynamic viscosity of the fluid respectively, **p** (Pa) is pressure and **f** (m.s-2) is an external acceleration field e.g. gravity.

From the Navier-Stokes equation (Equation 1), it can be seen that flow behaviour is directly determined by two key fluid properties- density and dynamic viscosity. CFD studies of *in vitro* systems typically model culture media as water at room temperature, where density and viscosity is assumed to be anywhere between 0.998-1000 kg/m^3 and 0.8-1 mPa.s respectively [29-33]. A second assumption is that these fluid properties are constant [23, 34, 35], which is only applicable for continuous flow or single throughput systems where cells constantly receive fresh medium. Culture media can be expected to denser and more viscous than water due to its higher solute content (sugars, inorganic salts, sera proteins). Furthermore, these properties intuitively change over the course of an experiment as cells absorb nutrients, growth factors and proteins, secrete extracellular matrix proteins and excrete metabolic by-products [36], which changes the solute content and therefore the density, viscosity and hydrodynamics of the media (Figure 1). The degree and rate of change is a function of cell type, initial number of seeded cells, proliferation rate, the type and quantities of secreted proteins and other experimental factors such as the initial volume of medium and the rates of medium recirculation and replenishment.

Given that the accuracy of any CFD simulation is directly determined by the assumptions, mathematical models, boundary conditions and fluid properties assigned during the setup of the model, it is important to use study-specific properties of culture media for more accurate CFD analysis of *in vitro* culture systems. It is understood that not all researchers have ready access to a rheometer or viscometer. Therefore, the densities and dynamic viscosities of two widely used commercial culture media – Dulbecco's Modified Eagle Medium (DMEM) and RPMI-1640 supplemented with 0, 5, 10 and 20% (v/v) foetal bovine serum (FBS) - were measured to serve as a reference for sera-free media (0%) and media supplemented with concentrations of FBS typically used for cell culture. This study also sought to investigate whether the properties of culture medium change during routine cell culture and found that

both the density and viscosity of the medium increased over three days of standard passaging of HN6 human head and neck and NCI-H460 human lung carcinoma cell lines, where differences between the results demonstrate cell-line specificity. CFD analysis of a simple microfluidic device design was conducted to investigate and compare any differences between modelling culture medium as water versus using experimentally-measured properties of culture medium.

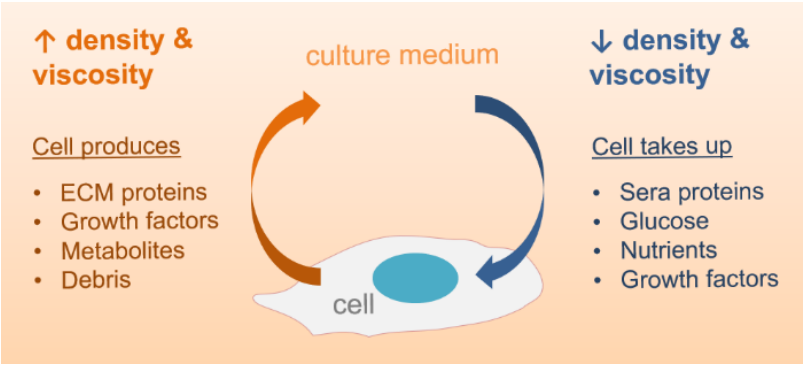


Figure 1. The net fluid properties of culture medium are determined by the mass transport balance between cells and medium

Gravimetric and rheological measurements were conducted to determine the density, dynamic viscosity and shear stress-rate profile (fluid behaviour) of the RPMI-1640 media supplemented with 5, 10 and 20% v/v foetal bovine serum (FBS) to model the range of FBS concentrations typically used in cell culture. To determine any changes in fluid properties of culture media during culture and demonstrate cell-line (model) specificity, the properties of 5% FBS-supplemented RPMI medium cultured with two different cell lines, NCI-H460 and HN6 were measured. Distinct properties were determined for each FBS-supplemented medium, where dynamic viscosity and density increased directly proportional to the concentration of added sera proteins. The dynamic viscosity and density of the 5% FBS-supplemented RPMI medium significantly increased after routine culture. Preliminary CFD analysis through a simple microfluidic channel using these experimentally-derived input values revealed differences in the fluid shear stress profiles and magnitude of wall shear stresses generated by the different fluid properties. Overall, these results recommend the use of experimen characterizing model-specific fluid properties of model-specific culture media for optimized design and more accurate CFD analysis of *in vitro* culture systems.

2. Materials & Methods

2.1 Sample preparation & cell culture

10mL samples of RPMI-1640 (Gibco, Thermo Fisher Scientific) and DMEM high glucose medium (11965-092, Gibco, Thermo Fisher Scientific) supplemented with 0, 5, 10 and 20 % v/v FBS (10091148, Gibco, Thermo Fisher Scientific) were dispensed into 10mL capped tubes. To evaluate the changes in the density and viscosity of culture medium during cell culture, 10 mL samples were extracted during routine passaging of NCI-H460 and HN6 cells after 3 days of culture. Both cell lines were passaged with an initial seeding density of approximately 2 x 10^6 cells per T75 flask and grown in 12 mL of RPMI-1640 medium supplemented with 5% FBS in a standard incubator (37°C and 5% CO_2). All media samples were kept refrigerated then warmed up to 37 ° C in a water bath prior to measurement. Deionized water (ddH_2O) and 1X phosphate buffer solution (PBS) controls were also prepared.

2.2 Density measurement

1 mL samples were initially dispended in a dish using a standard 1000µL pipette and weighed on a digital laboratory scale (Sartorius BP210D, Goettingen, Germany). However, small droplets of culture media were found to adhere to the inner surface of the pipette tips which compromised the accuracy of the results. Therefore, samples were measured subtractively. Briefly, individual sample tubes were placed on the scale, which was then tared. The tube was removed and vortexed for 5 seconds to ensure even solute distribution, then 1mL was removed from the tube using the 1000µL pipette. The sample tube was then re-weighed on the tared scale and the resultant weight difference was recorded. The 1mL samples were dispensed into a second tube for rheological measurement. Ten measurements were taken for each sample, using the same pipette for consistency. The fluid density for each sample was then calculated by dividing the weight values by 1 cm^3, then averaged, collated and plotted for comparison.

2.3 Rheology

Fresh FBS-supplemented DMEM and RPMI-1640 culture media and spent media samples were prepared as in Section 2.1 along with deionized water controls. A rheometer (Paar Physica MCR 300 Modular Compact Rheometer, Anton Paar GmbH, Austria) was set up with parallel 50 mm diameter stainless steel plates at a horizontal position (0°) and z-gap of 0.5 mm to define a sample volume of 0.981 mL. An adjunctive temperature control chamber was set at 37 ^{o} C. 1mL samples were individually loaded onto the centre of the lower platen, verified to be free of bubbles, then the top plate was lowered onto the sample. Sample fluid exceeding the test volume was trimmed with tissue paper. Rotational shear was then applied from 0 – 100 1/s. Measurements were conducted at 37° C with a minimum of 6 replicates per sample. The plates were thoroughly cleaned with 70% ethanol solution between tests and deionized water controls were used to calibrate the system between sample changes. Data was recorded on RheoCompass then exported for analysis. The datasets for each sample were plotted as viscosity profiles and shear stress-shear rate plots. The dynamic viscosity of each sample was then calculated by extracting the gradients of the shear-stress-shear plots.

2.4 Computational Fluid Dynamics

CFD simulations were conducted to determine any differences in flow mechanics within a simple microfluidics channel produced using experimentally-derived fluid properties for culture media compared to modelling with the properties of water. For this study, the values measured for fresh RPMI-1640 medium supplemented with 0, 5 and 10% v/v FBS and spent medium (originally RPMI-1640 supplemented with 5% FBS) after 3 days of culturing NCI-H460 and HN6 cells were modelled. These concentrations were selected to represent sera-free media and the most commonly used concentration of FBS, as well as to determine any differences in flow properties during/after cell culture. Briefly, a simple bifurcated microfluidic channel model was created on SolidWorks version 2019 then imported into ANSYS Fluent version 2020 R2 for analysis. A tetrahedral dropped node mesh was generated from the channel geometry, which represents the fluid domain, and mesh refinement was performed to ensure adequate discretisation. A mesh quality of 0.33 was confirmed in ICEM. The following assumptions were made and applied: (i) culture medium is an incompressible generalized Newtonian fluid, (ii) steady state fully laminar flow (Reynold's number < 2000), (iii) flow occurs at 37° C at atmospheric pressure and a gravitational acceleration of $9.81 m.s^{-2}$ and (iv) non-slip wall boundaries. A steady state viscous laminar model was selected with gravitational acceleration of $9.81 m/s^2$ and a pressure-based solver at gauge pressure. The inlet was set as a velocity inlet with directional flow defined via Cartesian co-ordinates in the negative Y direction (downwards into the fluid domain).

The inlet of the channel was set as a velocity inlet with a flow rate of 1.2 x 10^{-6} m.s^{-1} to model the average physiological capillary perfusion rate [37] and the outlet was set at gauge pressure without backflow. The convergence criterion was set at 10^{-7} for continuity, x, y and z-velocities.

To model different culture media solutions, custom entries were created in the materials library using the density and viscosity values measured (Sections 3.1, 3.2). For this study, 5% and 10% v/v FBS-supplemented RPMI-1640 media and spent media after culturing cells (originally 5% FBS-supplemented RPMI-1640) were modelled, along with default water properties and water at 37°C (Table). The cell zone was set to 'fluid', and the experimentally-derived culture medium properties were assigned. After setting up the model, material properties were applied for each fluid simulation as summarised in Table 1 as follows:

Table 1. Properties assigned to simulate water and different culture media formulations in a microfluidics channel

Material	Density (kg/m^3)	Viscosity (kg/m.s)
Water (default in ANSYS)	998.2	1.003 x 10^{-3}
Water at 37°C	993.3	0.6913 x 10^{-3}
RPMI + 0% FBS	999.3	0.733 x 10^{-3}
RPMI + 5% FBS	1002	0.848 x 10^{-3}
RPMI + 10% FBS	1007	0.958 x 10^{-3}
Spent RPMI (NCI-H460)	1015	0.954 x 10^{-3}
Spent RPMI (HN6)	1013	1.086 x 10^{-3}

Simulations were then performed with a maximum of 1000 iterations or until convergence. Using CFD-Post, plots of wall shear stress, global pressure, velocity vectors and flow path lines were generated to better visualise flow field properties.

3. Results & Discussion

3.1 Gravimetric density of culture media

The density of a material is a defined as its mass per unit volume by the formula ρ = mass/volume, with units expressed in g/cm^3 or kg/m^3 (1 mL = 1 cm^3 for fluids). Density is influenced by temperature and pressure, which affects the volume and molecular state of a material. As the intended operational conditions for the majority of *in vitro* devices occur at atmospheric pressure (1 atm) and 37 ^{o} C, the culture media samples and controls were measured under these conditions.

3.1.1 Methodology

The subtractive mass measurement approach was straightforward and addressed the issue of uncontrolled droplet retention within the pipette tip initially encountered during dispensed sample mass measurement. The charge density of the proteins, salts and sugars present in the media samples likely contributed to higher surface tension which led to sample retention within the pipette tips. Otherwise, no droplets were observed on the outer surface of the tips when drawing samples during subtractive weighing. This method is therefore recommended for measuring the density of more viscous fluid samples such as culture medium.

3.1.2 Results

The densities of the culture media samples and controls are presented in Figure 2 and Table 2 as follows:

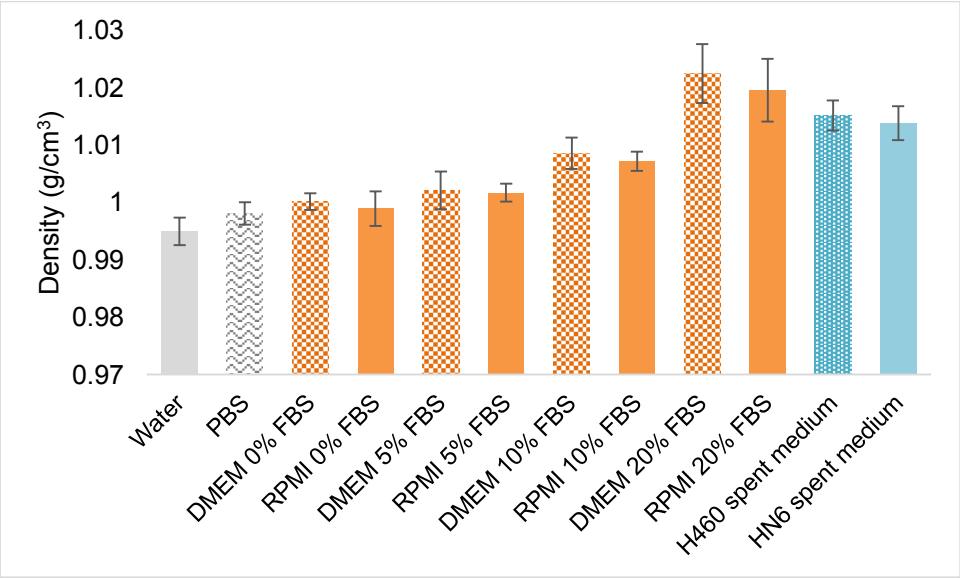


Figure 2. The density of DMEM (high glucose) and RPMI-1640 media supplemented with 0, 5, 10, 20 % v/v FBS and 5 % FBS-supplemented RPMI-1640 media after 3 days of standard subculture of H460 and HN6 cell lines. Deionized water and PBS were measured as controls. Values are expressed as mean ± standard deviation.

Table 2. Density and dynamic viscosity values measured for each sample

	Density (g/cm^3)		**Dynamic Viscosity (mPa.s)**	
%FBS (v/v)	DMEM (high glucose)	RPMI-1640	DMEM (high glucose)	RPMI-1640
0	1.000 ± 0.001	0.999 ± 0.003	0.731 ± 0.015	0.733 ± 0.006
5	1.002 ± 0.003	1.002 ± 0.002	0.862 ± 0.029	0.848 ± 0.021
10	1.009 ± 0.003	1.007 ± 0.002	0.930 ± 0.034	0.958 ± 0.032
20	1.023 ± 0.005	1.020 ± 0.005	1.050 ± 0.027	1.089 ± 0.044
Water		0.995 ± 0.002	0.659 ± 0.017	
PBS		0.998 ± 0.002	N/A	
H460 spent medium		1.015 ± 0.003	0.954 ± 0.070	
HN6 spent medium		1.013 ± 0.003	1.086 ± 0.073	

3.1.3 Discussion

The density of water at 37 ° C is 0.9933 $g.cm^{-3}$ (IAPWS R12-08), thus the value measured (0.995 $g.cm^{-3}$) for the deionized water control is within acceptable calibration standards for the 1000 µL pipette (Gibson). There was no significant difference between the densities measured for the culture media solutions compared to water (Figure 2). Nevertheless, all culture media samples and 1X PBS were found to have higher densities than deionized water, which was as expected due to the presence of salts, sugars and other solutes within these solutions. The density of both DMEM and RPMI-1640 medium increased directly proportional to the % (v/v) of added FBS (Figure 2), which is as expected given that FBS is the largest % volume additive in complete media and the main component of FBS is bovine serum albumin, a heavy, high molecular weight protein. High glucose DMEM, which contains 4.5 g/L of glucose, was

consistently found to have higher density than RPMI-1640, which contains 2.0 g/L glucose. Otherwise, both media contain approximately 11 g of inorganic salts and the relatively smaller percentage volumes of other additives such as growth factors can be assumed to have negligible effect on fluid properties.

The net density of 5% FBS-supplemented RPMI-1640 media increased by 1.297% and 1.197% after culturing NCI-H460 and HN6 cell lines for 3 days respectively, supporting the axiomatic hypothesis that general cellular activities including metabolism, secretion of ECM proteins, factors, signalling molecules, waste products and debris increase the net density of culture medium during culture. Differences between the results for the NCI-H460 and HN6 cell lines can be attributed to cell type-specific metabolic and proliferation rates, as well as the quantity, type, size and other properties of the proteins secreted by each respective cell line. While not numerically significant, this increase in fluid density equates to higher hydrostatic pressure P as given by $P = \rho gh$, where ρ is the density, g denotes the gravitational constant and h is the depth of the fluid. Any changes in pericellular pressure may be significant or is at least perceived on a cellular level, but it is likely that changes in osmotic flux and oxygen diffusion due to increased ionic concentration from cellular secretion would have a greater effect on cellular responses. The density values were then applied in preliminary CFD analysis of a typical organ-on-chip microfluidics channel.

3.2 Rheological properties of culture medium

Rheometry was performed to measure the dynamic viscosity of the culture media samples. Viscosity corresponds to the informal concept of 'thickness' and provides a measure of the resistance of a fluid to deformation or flow with application of shear or tensile stress, as defined by the following equation:

$$viscosity\ \mu = \frac{shear\ stress}{strain\ rate}\ (Pa.s)$$

Viscosity is a quantity expressing the magnitude of internal friction within a fluid, i.e. intermolecular friction between neighbouring particles that are moving at different velocities. The size, shape and magnitude of intermolecular forces of particles within a fluid determine its viscosity. As cell cultures proliferate, metabolic activity and other processes can be expected to increase the number of solute particles in culture media, thereby increasing its viscosity. For the majority of liquids, viscosity decreases with increasing temperature and increases with higher pressure as inter-particle energies are dependent on temperature and pressure. Therefore, samples were analysed at 37° C and 1 atm for model fidelity as for density measurement.

3.2.1 Methodology

The protocol described is standard for conducting rheometry with a parallel plate configuration as appropriate for low to medium viscosity fluids. A faint residue was observed on the plates after each sample measurement, indicating potential adsorption of proteins and molecules onto the test apparatus which may affect the consistency of the results. Although care was taken, other potential sources of error include the presence of microbubbles introduced during sample loading, inconsistent sample volume in the order of μL, test temperature stability and accidental contamination of the samples with particulate matter such as dust.

3.2.2 Results

The dynamic viscosities of each sample are presented in Figure 3 as follows.

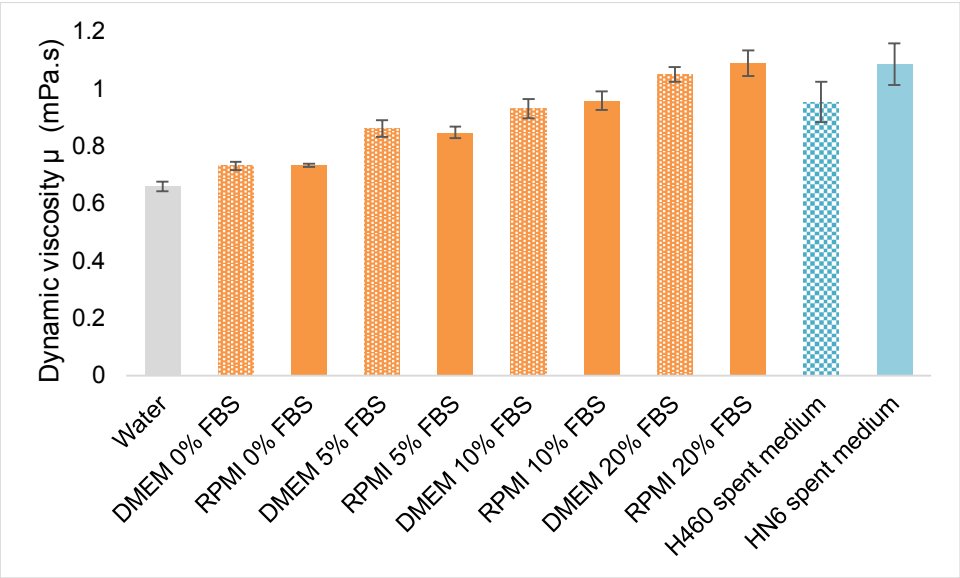


Figure 3. Dynamic viscosities of DMEM (high glucose) and RPMI-1640 media supplemented with typical concentrations of FBS (0, 5, 10, 20% v/v) and 5% FBS-supplemented RPMI-1640 after 3 days of standard subculture of NCI-H460 and HN6 cell linses. Samples were measured at 37°C along with a deionized water control. Values are expressed as mean ± standard deviation. Significance (p value > 0.05) as shown.

Table 3. Dynamic viscosities of culture media and % increase compared to water

Sample (at 37°C)	Dynamic viscosity μ (mPa.s)	% increase compared to water
Water	0.659 ± 0.017	0
DMEM + 0% FBS	0.731 ± 0.015	10.924
DMEM + 5% FBS	0.861 ± 0.029	30.706
DMEM + 10% FBS	0.930 ± 0.034	41.069
DMEM + 20% FBS	1.050 ± 0.027	59.250
RPMI-1640 + 0% FBS	0.733 ± 0.006	11.138
RPMI-1640 + 5% FBS	0.848 ± 0.021	28.597
RPMI-1640 + 10% FBS	0.958 ± 0.032	45.255
RPMI-1640 + 20% FBS	1.089 ± 0.044	65.135
NCI-H460 (RPMI-1640 + 5% FBS)	0.954 ± 0.070	44.619
HN6 (RPMI-1640 + 5% FBS)	1.086 ± 0.074	64.656

3.2.3 Discussion

Culture medium is frequently assumed to be a generalized Newtonian fluid in CFD analyses of *in vitro* culture systems [4, 25, 34, 38]. Newtonian fluids have a constant viscosity that is independent of shear stress and strain rate, which is exhibited as a linear trend on shear stress-shear rate plots and viscosity-shear profiles. Linear trends (R^2 values > 0.995) were obtained for all media samples including the deionized water controls (Supplementary Data), indicating that DMEM and RPMI-1640 media supplemented with typical concentrations of FBS can be considered as Newtonian fluids for rheological and computational modelling purposes. However, slight shear thinning was observed on viscosity-shear rate profiles at lower shear rates (0-20/s), which became more pronounced with higher concentrations of added FBS (Supplementary data/available upon request). While this could be due to measurement instability at lower shear rates, this is consistent with the shear thinning behaviour of many

protein-rich biological fluids such as blood plasma [39], and warrants consideration for CFD analysis. Higher concentrations of FBS or addition of viscosity modifiers such as dextran may produce non-Newtonian fluid behaviours due to the effects of shear-induced polymer chain alignment [40].

The average dynamic viscosity of the deionized water control was measured to be 0.659 mPa.s (Figure 3, Table 2), which was lower than the 0.6913 mPa.s standard for water at 37°C (IAPWS R12-08). The difference is not statistically significant and may be potentially due to thermal overshooting within the temperature control chamber i.e. the actual test temperature may have been closer to 38°C instead of 37°C, where higher temperatures corresponds to lower viscosity for water. Nevertheless, distinct viscosities were measured for all samples. All culture media samples were confirmed to be more viscous than the deionized water control, where dynamic viscosity increased proportional to the % volume of added FBS as expected and corroborates density results (Figure 2). Even a 5% (v/v) addition of FBS, the lowest concentration, yielded a 30% increase in viscosity for both culture media compared to water at the same temperature, while the viscosities of 10% and 20% FBS-supplemented RPMI media was found to be 48% and 107% higher than that of the water control respectively (Table 3). Importantly, 5% FBS-supplemented RPMI media significantly increased by 12.084% and 39.220% after 3 days of culture of NCI-H460 and NH6 cell lines respectively (Table 3). This change can be significant on a cellular level, where viscosity impacts molecular diffusion kinetics (passive mass transport) and higher fluid viscosity will exert higher hydrostatic forces in and shear stresses in flow culture devices such as organ-on-chips, which affect cell motility, cell-substrate interactions and migration [41].

The magnitude and rate of change is affected by numerous experimental factors, including initial seeding density, duration of culture, the substrate, media formulation (e.g. addition of growth factors), the geometry of the culture domain, flow conditions, % recirculation as well as the location of cells within the system. In addition, there is an interdependency where the density and viscosity of culture media directly affects the diffusivity of soluble molecules and determines the degree of hydrostatic pressure (mechanical stimuli) exerted by the fluid environment on cells, which affect their function. Cell-line specific characteristics will also determine the viscosity of the culture medium, including the quantity and types of proteins secreted and mechanosensitivity to the effects of shear in flow-culture devices. Together, these factors collectively determine the diffusion profile of metabolites and secreted ECM proteins conceptually illustrated in Figure 1.

In the case of continuous perfusion of fresh medium, diffusion kinetics becomes a function of the flow rate, whereas in systems where medium is recirculated, recirculation introduces temporal effects where the balance between continual depletion of nutrients and increasing concentrations of metabolites both affect and occur as a function of the proliferation rate. In general, fluid flow reduces stagnant regions where heavier proteins and solutes can sink or aggregate and therefore generates more homogeneous fluid density. Furthermore, introduction of perfusion provides mechanical stimuli and improves mass transport, factors which are known to enhance the rate of cellular growth and proliferation [32, 42], axiomatically resulting in higher secretion of factors and proteins and hence the physical properties of culture media. This must be experimentally verified for each system. Finally, supplier and batch-to-batch variations of FBS may affect the consistency of the results (although overall trends can be expected to be similar), thus further recommending study- specific characterization of culture medium.

Overall, these results show that the dynamic viscosities of DMEM and RPMI-1640 media supplemented with conventional concentrations of FBS are significantly higher than that of water at 37°C. Given that viscosity is a key property that determines fluid behaviour, these results recommend using experimentally-derived fluid properties for CFD analysis where possible. Differences in hydrodynamics generated by these experimentally derived culture medium properties were compared to water by CFD analysis in the following section.

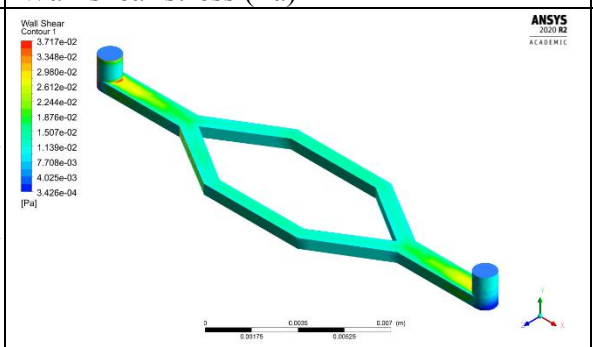
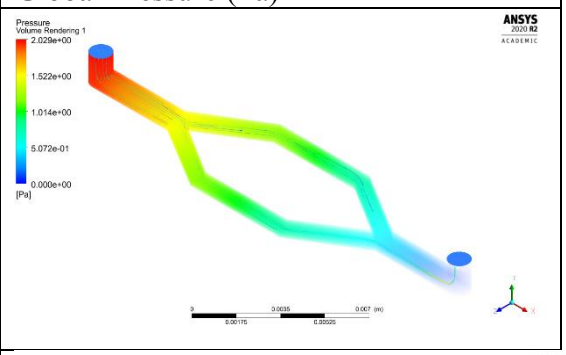
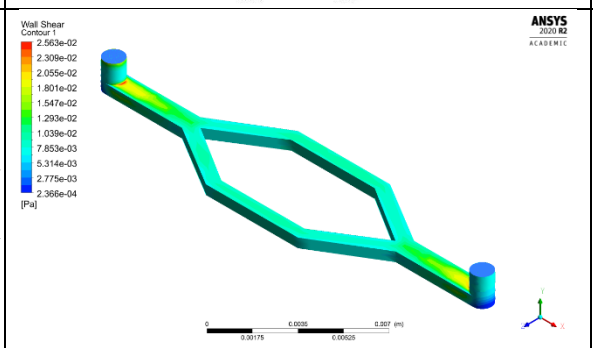
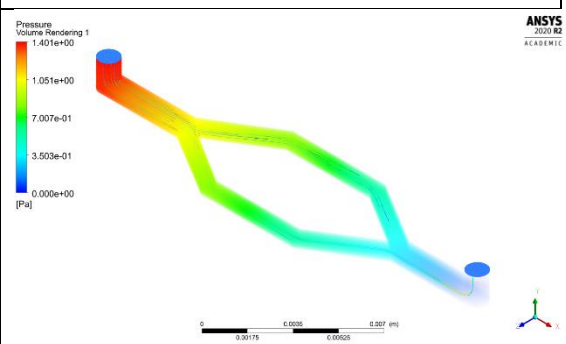
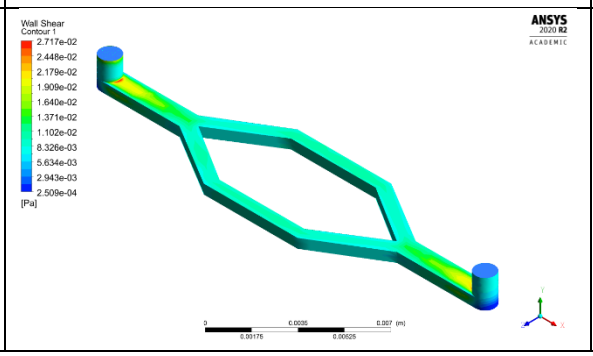
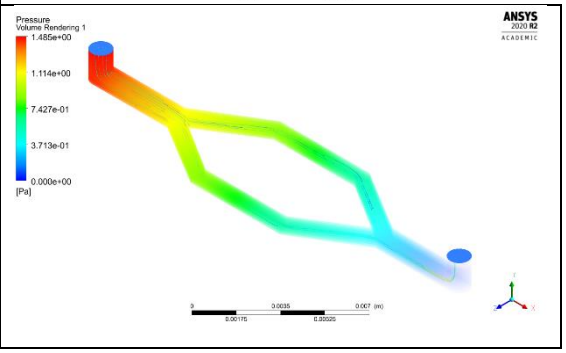
3.3 Computational Fluid Dynamics

CFD simulations were performed to examine and compare any differences between models that apply the default properties of water typically provided in ANSYS versus using the actual properties of water at 37 °C and experimentally-derived density and viscosity values for culture media. Results and implications for cells were will be discussed in the following section.

3.3.1 Results

The wall shear stress and global pressure profiles across the channel model and maximum values of these variables are presented and summarised in Tables 4 & 5 as follows:

Table 4. Wall shear stress and global pressure distributions within the channel model

	Wall shear stress (Pa)	Global Pressure (Pa)
Water (default)		
Water (37°C)		
RPMI + 0% FBS		

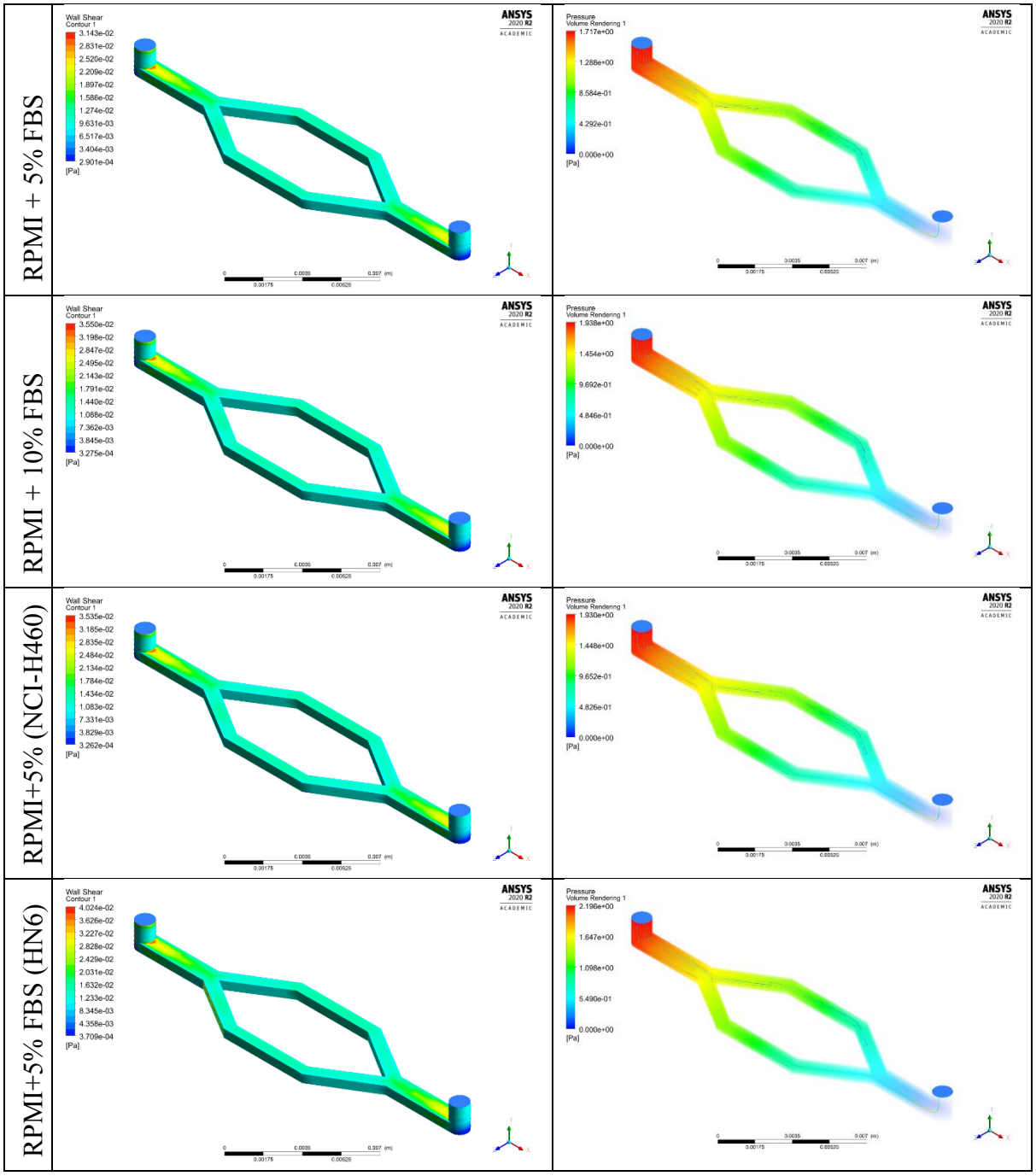


Table 5. Maximum wall shear stress and pressure for each model

Model	Max Wall Shear Stress (mPa)	Max Pressure (Pa)
Water (default)	37.17	2.029
Water (37°C)	25.63	1.401
RPMI-1640 + 0% FBS	27.17	1.485
RPMI-1640 + 5% FBS	31.43	1.717
RPMI-1640 + 10% FBS	35.50	1.938
Spent RPMI-1640* (H460)	35.35	1.930
Spent RPMI-1640* (HN6)	40.24	2.196
		*supplemented with 5% FBS

3.3.2 Discussion

As can be seen in the shear stress profiles (Table 4), the location of the maximum wall shear stress occurred at the inner junction between the inlet and outlet ports (cylindrical features) and the channel for all models at the flow rate investigated; this was as expected given that the flow rate is relatively low and the fluid properties measured are sufficiently similar (Table 2). Likewise, the global pressure profiles were consistent across all models given due to the flow conditions originally assigned. The maximum wall shear stress and global pressure increased in proportion to the dynamic viscosity and density of the fluid in accordance with the Navier Stokes equations (Equation 1), where higher % volumes of FBS (higher viscosity) directly corresponded with higher maximum shear and pressure within the channel (Table 2, Table 5). The maximum wall shear stress of 5% FBS supplemented RPMI-medium increased by 12.5% and 28.0% after 3 days of culturing NCI-H460 and HN6 cell lines respectively (Table 5). This result highlights the importance of determining model-specific fluid properties, particularly for analysing systems where culture medium is recirculated. Interestingly, the default properties of water in ANSYS yielded results similar to those of RPMI-1640 medium supplemented with 10% FBS and the spent medium (Table 5) as the viscosity was closer to values measured for the media with at least 10% v/v added FBS and the spent media (Table 1, Table 2). While this indicates that computational studies that have applied default water properties (typically at room temperature) in simulation programs can still be considered to be valid, the accuracy of any CFD model depends on inputs. Hence it is ideal to use actual experimentally-derived properties of culture medium, such as those measured in this work. For further model accuracy, a time course and measuring the surface roughness of substrate materials e.g. polycarbonate, glass, PDMS are recommended.

Conclusions

This study sought to measure the dynamic viscosity and density of common media formulations to serve as a ballpark reference for CFD analysis of cell and tissue culture devices. In this work, the fluid properties of high glucose DMEM and RPMI-1640 media supplemented with typical concentrations of FBS (0, 5, 10 and 20% v/v) were measured. RPMI-1640 media after 3 days of culture of two cell lines was also measured as a preliminary investigation of how the fluid properties of culture medium change during cell culture. The densities and dynamic viscosities of all media samples were definitively shown to be higher than that of deonized water at 37°C, where higher concentrations of added FBS directly correlated to higher density and dynamic viscosity of culture media as expected. Importantly, the density and viscosity of 5% FBS-supplemented RPMI-1640 was shown to increase after 3 days of culture of NCI-H460 and HN6 cell lines, where distinct differences between the results for each cell line indicate cell line and model specificity. As previously discussed, the magnitude and rate of change varies between cell types and numerous other factors including initial seeding density. For this reason, a time course to determine changes in the viscosity and density of culture medium was beyond the scope of this study. However, it is recommended and will be performed for validation studies.

CFD simulations of physiological flow through a simple organ-on-chip channel using the density and viscosity values measured demonstrated differences in the magnitudes of wall shear stress and global pressure between different media formulations (0, 5, 10% added FBS) and a water model, where wall shear stress and pressure increased with higher concentrations of added FBS (higher viscosity). The maximum wall shear stress and pressure significantly increased after 3 days of culture of NCI-H460 and HN6 cell lines, bearing implications for systems that recirculate medium. Applying the default values for water yielded results similar to those of RPMI-1640 medium supplemented with at least 10% FBS. Therefore, studies that have modelled culture medium as water and applied density and dynamic values within the

ranges measured can still be considered as valid. Nevertheless, these results highlight the importance of using model-specific fluid properties for CFD analysis and recommend the use of experimentally-derived properties of culture media for computational analysis of cell and tissue culture devices. It is hoped that the values measured for DMEM and RPMI-1640 media will serve as a useful baseline reference for anyone conducting such analyses of cell or tissue culture devices, particularly those where culture media is recirculated for any duration of culture. Overall, this work recommends determining both baseline and temporal fluid properties of culture media for more accurate CFD analysis and optimized design of *in vitro* culture systems.

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References

[1] T. Yao and Y. Asayama, "Animal-cell culture media: History, characteristics, and current issues," (in eng), *Reproductive medicine and biology,* vol. 16, no. 2, pp. 99-117, 2017.

[2] A. Sen, M. S. Kallos, and L. A. Behie, "Expansion of mammalian neural stem cells in bioreactors: effect of power input and medium viscosity," *Developmental Brain Research,* vol. 134, no. 1–2, pp. 103-113, 2002.

[3] J. L. Moreira *et al.*, "Effect of Viscosity upon Hydrodynamically Controlled Natural Aggregates of Animal Cells Grown in Stirred Vessels," *Biotechnology Progress,* vol. 11, no. 5, pp. 575-583, 1995.

[4] S. H. Cartmell, B. D. Porter, A. J. García, and R. E. Guldberg, "Effects of Medium Perfusion Rate on Cell-Seeded Three-Dimensional Bone Constructs in Vitro," *Tissue Engineering,* vol. 9, no. 6, pp. 1197-1203, December 2003 2003.

[5] R. Pörtner and C. Giese, "An Overview on Bioreactor Design , Prototyping and Process Control for Reproducible Three-Dimensional Tissue Culture," no. 3, pp. 53-78, 2007.

[6] J. Rouwkema, S. Gibbs, M. P. Lutolf, I. Martin, G. Vunjak-Novakovic, and J. Malda, "In vitro platforms for tissue engineering: implications for basic research and clinical translation," *Journal of tissue engineering and regenerative medicine,* vol. 5, no. 8, pp. e164-e167, 2011.

[7] W. J. Polacheck, R. Li, S. G. M. Uzel, and R. D. Kamm, "Microfluidic platforms for mechanobiology," (in eng), *Lab on a chip,* vol. 13, no. 12, pp. 2252-2267, 2013.

[8] H. W. Hou, W. C. Lee, M. Leong, S. Sonam, S. Vedula, and C. T. Lim, "Microfluidics for Applications in Cell Mechanics and Mechanobiology," *Cellular and Molecular Bioengineering,* vol. 4, pp. 591-602, 01/01 2012.

[9] F. Kurth, K. Eyer, A. Franco-Obregón, and P. S. Dittrich, "A new mechanobiological era: microfluidic pathways to apply and sense forces at the cellular level," *Current Opinion in Chemical Biology,* vol. 16, no. 3, pp. 400-408, 2012/08/01/ 2012.

[10] E. Ergir, B. Bachmann, H. Redl, G. Forte, and P. Ertl, "Small Force, Big Impact: Next Generation Organ-on-a-Chip Systems Incorporating Biomechanical Cues," (in English), *Frontiers in Physiology,* Mini Review vol. 9, no. 1417, 2018-October-09 2018.

[11] J. M. Osborne, R. D. O'Dea, J. P. Whiteley, H. M. Byrne, and S. L. Waters, "The Influence of Bioreactor Geometry and the Mechanical Environment on Engineered Tissues," *Journal of Biomechanical Engineering,* vol. 132, no. 5, 2010.

[12] S. G. Mina, W. Wang, Q. Cao, P. Huang, B. T. Murray, and G. J. Mahler, "Shear stress magnitude and transforming growth factor-βeta 1 regulate endothelial to mesenchymal transformation in a three-dimensional culture microfluidic device," *RSC Advances,* 10.1039/C6RA16607E vol. 6, no. 88, pp. 85457-85467, 2016.

[13] J. M. Barnes, J. T. Nauseef, and M. D. Henry, "Resistance to Fluid Shear Stress Is a Conserved Biophysical Property of Malignant Cells," *PLOS ONE,* vol. 7, no. 12, p. e50973, 2012.

[14] G. Kretzmer and K. Schügerl, "Response of mammalian cells to shear stress," *Applied Microbiology and Biotechnology,* vol. 34, no. 5, pp. 613-616, 1991/02/01 1991.

[15] W. Yu *et al.*, "A Microfluidic-Based Multi-Shear Device for Investigating the Effects of Low Fluid-Induced Stresses on Osteoblasts," *PLOS ONE,* vol. 9, no. 2, p. e89966, 2014.

[16] F. Tovar-Lopez *et al.*, "A Microfluidic System for Studying the Effects of Disturbed Flow on Endothelial Cells," (in English), *Frontiers in Bioengineering and Biotechnology,* Brief Research Report vol. 7, no. 81, 2019-April-17 2019.

[17] J. G. Santiago, S. T. Wereley, C. D. Meinhart, D. J. Beebe, and R. J. Adrian, "A particle image velocimetry system for microfluidics," *Experiments in Fluids,* vol. 25, no. 4, pp. 316-319, 1998/09/01 1998.

[18] A. Campos Marin, T. Grossi, E. Bianchi, G. Dubini, and D. Lacroix, "2D μ-Particle Image Velocimetry and Computational Fluid Dynamics Study Within a 3D Porous Scaffold," *Annals of Biomedical Engineering,* vol. 45, no. 5, pp. 1341-1351, 2017/05/01 2017.

[19] H.-S. Chuang and Y.-L. Lo, "Microfluidic velocity measurement using a scanning laser Doppler microscope," *Optical Engineering,* vol. 46, no. 2, p. 024301, 2007.

[20] Y. S. Morsi, W. W. Yang, A. Owida, and C. S. Wong, "Development of a novel pulsatile bioreactor for tissue culture," (in eng), *J Artif Organs,* vol. 10, no. 2, pp. 109-14, 2007.

[21] L. Stern *et al.*, "Doppler-based flow rate sensing in microfluidic channels," (in eng), *Sensors (Basel, Switzerland),* vol. 14, no. 9, pp. 16799-16807, 2014.

[22] J. D. Salvi, J. Y. Lim, and H. J. Donahue, "Finite Element Analyses of Fluid Flow Conditions in Cell Culture," *Tissue Engineering. Part C, Methods,* vol. 16, no. 4, pp. 661-670, 2010.

[23] D. Freitas, H. A. Almeida, and P. J. Bártolo, "Perfusion Bioreactor Fluid Flow Optimization," *Procedia Technology,* vol. 16, pp. 1238-1247, 2014/01/01/ 2014.

[24] T. Glatzel *et al.*, "Computational fluid dynamics (CFD) software tools for microfluidic applications – A case study," *Computers & Fluids,* vol. 37, no. 3, pp. 218-235, 2008/03/01/ 2008.

[25] M. Huang, S. Fan, W. Xing, and C. Liu, "Microfluidic cell culture system studies and computational fluid dynamics," *Mathematical and Computer Modelling,* vol. 52, no. 11, pp. 2036-2042, 2010/12/01/ 2010.

[26] A. Marturano-Kruik *et al.*, "Human bone perivascular niche-on-a-chip for studying metastatic colonization," *Proceedings of the National Academy of Sciences,* vol. 115, p. 201714282, 01/23 2018.

[27] A. R. Patrachari, J. T. Podichetty, and S. V. Madihally, "Application of computational fluid dynamics in tissue engineering," *Journal of bioscience and bioengineering,* vol. 114, no. 2, pp. 123-32, 2012.

[28] D. W. Hutmacher and H. Singh, "Computational fluid dynamics for improved bioreactor design and 3D culture," *Trends in biotechnology,* vol. 26, no. 4, pp. 166-72, 2008.

[29] M. Israelowitz, B. Weyand, S. Rizvi, P. Vogt, and H. von Schroeder, "Development of a Laminar Flow Bioreactor by Computational Fluid Dynamics," *Journal of Healthcare Engineering,* vol. 3, pp. 455-476, 09/01 2012.

[30] M. Cioffi, F. Boschetti, M. T. Raimondi, and G. Dubini, "Modeling evaluation of the fluid-dynamic microenvironment in tissue-engineered constructs: a micro-CT based model," *Biotechnology and Bioengineering,* vol. 93, no. 3, pp. 500-10, 2006.

[31] M. Malvè, D. J. Bergstrom, and X. B. Chen, "Modeling the flow and mass transport in a mechanically stimulated parametric porous scaffold under fluid-structure interaction approach," *International Communications in Heat and Mass Transfer,* vol. 96, pp. 53-60, 2018/08/01/ 2018.

[32] S. Sugiura, Y. Sakai, K. Nakazawa, and T. Kanamori, "Superior oxygen and glucose supply in perfusion cell cultures compared to static cell cultures demonstrated by simulations using the finite element method," *Biomicrofluidics,* vol. 5, no. 2, p. 022202, 2011.

[33] Y. Guyot, F. P. Luyten, J. Schrooten, I. Papantoniou, and L. Geris, "A Three-Dimensional Computational Fluid Dynamics Model Of Shear Stress Distribution During Neotissue Growth In A Perfusion Bioreactor," *Biotechnology and Bioengineering,* vol. 112, 06/01 2015.

[34] B. Porter, R. Zauel, H. Stockman, R. Guldberg, and D. Fyhrie, "3-D computational modeling of media flow through scaffolds in a perfusion bioreactor," *Journal of Biomechanics,* vol. 38, no. 3, pp. 543-9, 2005.

[35] S. P. Singh, M. Shukla, and R. K. Srivastava, "Lattice Modeling and CFD Simulation for Prediction of Permeability in Porous Scaffolds," *Materials Today: Proceedings,* vol. 5, no. 9, Part 3, pp. 18879-18886, 2018/01/01/ 2018.

[36] S. Yedgar, D. B. Weinstein, W. Patsch, G. Schonfeld, F. E. Casanada, and D. Steinberg, "Viscosity of culture medium as a regulator of synthesis and secretion of very low density lipoproteins by cultured hepatocytes," *Journal of Biological Chemistry,* vol. 257, no. 5, pp. 2188-2192, March 10, 1982 1982.

[37] F. Ye, S. Yin, M. Li, Y. Li, and J. Zhong, "In-vivo full-field measurement of microcirculatory blood flow velocity based on intelligent object identification," *Journal of Biomedical Optics,* vol. 25, no. 1, p. 016003, 2020.

[38] H. Stockman, "Lattice Boltzmann Method for Calculating Fluid Flow and Dispersion in Porous and Fractured Media," in *Gas Transport in Porous Media*, vol. 20, C. Ho and S. Webb, Eds. (Theory and Applications of Transport in Porous Media: Springer Netherlands, 2006, pp. 221-242.

[39] M. Brust, C. Schaefer, L. Pan, M. Garcia, P. Arratia, and C. Wagner, "Rheology of Human Blood Plasma: Viscoelastic Versus Newtonian Behavior," *Physical Review Letters,* vol. 110, p. 078305, 02/17 2013.

[40] M. A. Tung, "Rheology of Protein Dispersions," *Journal of Texture Studies,* vol. 9, no. 1- 2, pp. 3-31, 1978.

[41] J. Gonzalez-Molina *et al.*, "Extracellular fluid viscosity enhances liver cancer cell mechanosensing and migration," *Biomaterials,* vol. 177, pp. 113-124, 2018/09/01/ 2018.

[42] T. Kitagawa, T. Yamaoka, R. Iwase, and A. Murakami, "Three-dimensional cell seeding and growth in radial-flow perfusion bioreactor for in vitro tissue reconstruction," *Biotechnology and Bioengineering,* vol. 93, no. 5, pp. 947-954, 2006.