Improvement and validation of a computational model of flow in the swirling well cell culture model

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Abstract
Effects of fluid dynamics on cells are often studied by growing the cells on the base of cylindrical wells or dishes that are swirled on the horizontal platform of an orbital shaker. The swirling culture medium applies a shear stress to the cells that varies in magnitude and directionality from the center to the edge of the vessel. Computational fluid dynamics methods are used to simulate the flow and hence calculate shear stresses at the base of the well. The shear characteristics at each radial location are then compared with cell behavior at the same position. Previous simulations have generally ignored effects of surface tension and wetting, and results have only occasionally been experimentally validated. We investigated whether such idealized simulations are sufficiently accurate, examining a commonly-used swirling well configuration. The breaking wave predicted by earlier simulations was not seen, and the edge-to-center difference in shear magnitude (but not directionality) almost disappeared, when surface tension and wetting were included. Optical measurements of fluid height and velocity agreed well only with the computational model that incorporated surface tension and wetting. These results demonstrate the importance of including accurate fluid properties in computational models of the swirling well method.

KEYWORDS
endothelial cells, particle image velocimetry, synthetic PIV, transverse WSS, wall shear stress

1 INTRODUCTION

Fluid dynamic shear stresses are applied to cells in vitro to understand physiological and pathological processes. Effects of shear on vascular endothelial cells have received particular attention because spatial variation in haemodynamic wall shear stress (WSS) is thought to determine the nonuniform prevalence of atherosclerosis within the arterial tree. Endothelial cells have been exposed to shear stresses chiefly by using parallel-plate flow chambers or cone-and-plate devices. Such studies have shown that the applied shear stress can affect fundamental properties including cell shape, cytoskeletal organization, rates of mitosis, secretion of signaling molecules, and expression of numerous genes (see, e.g., Chien et al., 1998; Li et al., 2005; McCormick et al., 2003).

Both systems can apply unidirectional flow, or uniaxial flow that is oscillatory or pulsatile, and effects of these characteristics, as well as of the mean shear magnitude, have been determined (Barakat & Lieu, 2003; Li et al., 2005). However, neither can produce the truly
multidirectional flow that has recently been implicated in the localization of arterial disease (Mohamied et al., 2015). Furthermore, there are practical limitations to how long the shear can be applied; chronic application of flow, as occurs in vivo, is impracticable. To overcome this limitation, an increasing number of studies use the “swirling well” or “orbital shaker” method, in which cells are cultured on the base of a cylindrical dish or well while fluid motion is induced by placing the culture vessel on the horizontal platform of a shaker that translates in a circular orbit in the plane of the platform. The orbital motion forces a wave to swirl around the vessel; in broad terms, shear stress has lower magnitude and greater multidirectionality towards the center of the well than towards the edge. Local flow conditions are compared with local cell behavior. A recent review (Warboys et al., 2019) summarized 20 studies that have used this paradigm to investigate effects of shear on endothelial cells. Near the edge, cells demonstrate homeostatic properties such as elevated expression of eNOS and Krüppel-like factor 4 whereas cells towards the center exhibit potentially atherogenic behavior such as expression of intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin (reviewed in Warboys et al., 2019).

Because endothelial cells respond to the magnitude of the applied shear, the degree of its fluctuation, and changes in its direction, and because these factors have been implicated in triggering atherosclerosis, it is important to characterize the flow to which the cells are exposed with high accuracy. This is a more difficult problem for the swirling well than for the parallel-plate flow chamber and cone-and-plate devices. Due to the complexity of the flow within the well and the presence of a free surface, the complete fluid behavior cannot be derived analytically, as with the other two devices, although recent advances permit the main flow features—the presence or absence of wave breaking and resonance—to be predicted for different combinations of orbital velocity, orbital radius, medium height, and well diameter. They also permit shear stresses at different locations on the base of the well to be approximated, but there is necessarily a compromise between tractability and accuracy (Alpresa et al., 2018a, 2018b). Computational fluid dynamics (CFD) methods have therefore been used to model the flow and obtain the shear (Alpresa et al., 2018a; 2018b; Bai et al., 2012; Barrett et al., 2010; Berson et al., 2008; Chakraborty et al., 2012; Discacciati et al., 2013; Filipovic et al., 2016; Ghim et al., 2017; Kim & Kizito, 2009; Salek et al., 2012; Thomas et al., 2017; Velasco et al., 2016; Warboys et al., 2010; Warboys et al., 2014; Zhang et al., 2009). Only a few of these studies have included surface tension and wetting in the model, and have experimentally validated the solutions.

Neglecting surface tension has been justified by computing the dimensionless Bond (Bo) and Weber (We) numbers, which indicate, respectively, the relative importance of gravitational acceleration to surface tension and the relative importance of inertia to surface tension:

$$Bo = \frac{(\rho_{water} - \rho_{air}) g (2R)^2}{\sigma},$$  
$$We = \frac{\rho_{water} U^2 (2R)}{\sigma},$$

where $\rho$ is density, $U$ is linear velocity, $R$ is the radius of the well, $\sigma$ is the surface tension coefficient, and $g$ is gravitational acceleration (Salek et al., 2012). Values of both numbers have generally been $>1$.

Although $\rho$, $\sigma$, and $g$ do not vary between different studies, $U$ depends on the orbital shaker type and settings and $R$ varies with the type of cell culture vessel. When the well diameter is large and the rotational rate and orbital radius are high, surface tension is negligible; gravitational and inertial forces largely determine the shape of the free surface. With a decrease in scale or linear velocity, surface tension plays a more important role. The contact angle between the liquid–air interface and the wall of the well also needs to be considered. It depends not only on surface tension but also on wettability. For a water-air interface in contact with a hydrophobic material, angles are $>90^\circ$, and for a hydrophilic material they are $<90^\circ$, giving rise to concave and convex menisci, respectively.

Here we used CFD with and without surface tension plus wetting to model fluid flow in a configuration that has been used in several published studies (Arshad et al., 2021; Ghim et al., 2017, 2021; Potter et al., 2011, 2012; Warboys et al., 2010): cells cultured in a standard 12-well plate (or on a Transwell® filter insert in a six-well plate) on a shaker with an orbital radius of 5 mm and rotational rate of 150 rpm. Incorporating surface tension and wetting changed the computed flow regime from a breaking to a non-breaking wave and virtually abolished the center-to-edge change in shear magnitude, but not shear directionalility. The results were validated by experimental particle image velocimetry (PIV), which was in turn compared with numerical simulation of the PIV, and by optical measurement of fluid surface height. The results alter interpretations of the effects of shear characteristics on endothelial cell behavior derived from previous studies and show that more attention should be paid to surface tension and wetting in future simulations.

## 2 METHODS

### 2.1 Overview

The focus of the study was a comparison between experimental and numerical characterizations of flow in a swirling well. Experimental characterization included measuring the height of fluid at different locations in the swirling well and measuring flow velocities within it. Both were achieved optically, the former by measuring the absorbance of light passing vertically though the fluid and the latter by PIV. Numerical characterization involved models of the swirling well that differed only in whether they did or did not include surface tension plus wetting. These physical properties of the fluid were determined experimentally, using a tensiometer to assess surface tension and confocal microscopy of fluid containing a fluorescent dye to map the meniscus and hence obtain the contact angle. Discrepancies and their causes were investigated by applying numerical and experimental methods to different fluids and different orbital rotation rates, and by employing a hybrid technique in which the idealized outcome of PIV was predicted from the results of the flow simulations.
2.2 | Surface tension measurement

Surface tension was measured using a Wilhelmy plate tensiometer (KRUSS). The following samples were tested: distilled water; complete cell culture medium comprising Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS), penicillin, streptomycin, gentamicin, amphotericin, endothelial cell growth factor, and l-glutamine; Rhodamine B (RB) solution; Evans’ Blue Dye (EBD) solution; and ethanol. All measurements were made in triplicate at 37°C.

2.3 | Contact angle measurement

An inverted laser-scanning confocal microscope (Leica TCS SP5) was used to image the shape of the meniscus within the wells. RB or EBD was dissolved in distilled water at concentrations of 7.0 µg/ml and 14 mg/ml, respectively, to provide a fluorescent medium that could be detected by the confocal, and the solution was placed in the well. 561 nm excitation and 594–680 nm emission wavelengths were used for RB and 561 nm and 660–759 nm for EBD. The optical sectioning capability of the microscope was used to capture images parallel to the base of the well, from the bottom to the top of the fluid in 25-µm-thick slices. Because the area of each image (1.55 × 1.55 mm) was smaller than the diameter of the well, many such z-stacks were acquired sequentially across the diameter and merged to produce a composite image of the whole height of the fluid along a strip across the diameter and the other increasing, and contributing equally in the center. The precise curvature is defined by \( \rho \), \( S \), \( g \), and \( \theta \), which are incorporated into the two constants, \( C_1 \) and \( C_2 \).

\[
y = C_1 \exp(-C_2 x) + C_2 \exp(C_2 x),
\]

where \( C_1 = \left( \frac{1}{2} \sqrt{\frac{g}{S \rho \cot \theta}} \right) \) and \( C_2 = \sqrt{\frac{g \rho}{S}} \).

2.4 | CFD modeling

The motion of fluid in one well of a 12-well plate translating in a circular orbit on a horizontal platform was simulated using commercial CFD software. The geometry consisted of a cylinder having diameter 22.1 mm, height 10 mm and an open surface, filled with liquid to a mean depth, \( d \), of 2 mm (Figure S2). The remaining space was filled with air. The base and side walls of the cylinder were defined as no-slip boundary conditions and the open surface was defined as a pressure outlet (atmospheric pressure). The motion of fluid in the well was described by continuity and Navier-Stokes equations:

\[
\frac{\partial \rho u}{\partial t} + \nabla \cdot (\rho u u) = -\nabla p + \nabla \cdot (\mu \nabla u) + f,
\]

where \( u \) is the velocity, \( p \) is the fluid density, \( t \) is the time, \( \rho \) is the pressure, \( \nu \) is the kinematic viscosity, and \( f \) is the force acting on the fluid. The equations assume that the fluid is incompressible and Newtonian. They were converted to discrete finite difference equations and solved using STAR-CCM+ (version 11.02.010-r8).

An acceleration given by:

\[
[A \omega^2 \cos(\omega t), A \omega^2 \sin(\omega t), -9.81]
\]

was applied in the \( x \), \( y \), and \( z \) planes, respectively, with \(-9.81\) being the gravitational acceleration, \( A \) the orbital radius (5 mm) and \( \omega \) the angular velocity (15.7 rad/s).

A volume fraction, \( C \), of the reference phase (liquid) was specified for each grid cell. Grid cells filled with liquid had \( C = 1 \); those filled with air had \( C = 0 \), and cells containing the interface had values in the range \( 0 < C < 1 \). The free surface was defined as the isosurface where \( C = 0.5 \). Velocity and pressure were obtained using the segregated flow model, which solves the momentum and continuity equations sequentially in an uncoupled manner, linking them using a predictor-corrector approach. Physical properties of the various liquids incorporated into the computational models are given in Table 1. For all models, air was assumed to have density 1.1115 kg/m³ and kinematic viscosity 16.8 × 10⁻⁶ mm²/s.

The simulation was initiated from a static state. Due to the periodic nature of the flow within the well, the maximum and minimum shear stress at the base of the well was expected to reach a constant value after initial transient effects had dampened out. Physical time, divided into increments of \( 1 \times 10^{-6} \) s, was solved using an implicit unsteady scheme. Each time step was iterated until asymptotic convergence of the solution, defined as a < 1 × 10⁻⁵ Pa change over five iterations, was achieved. A Courant-Friedrichs-Lewy (CFL) number of <0.5 on average was maintained to satisfy the High-Resolution Interface Capturing (HRIC) scheme. Six hundred and fifty-two thousand mesh cells were used, as determined by a previous mesh convergence study (Alpresa et al., 2018a).
The "multiphase interaction model" was used to incorporate surface tension. Following Brackbill et al. (1992), the surface tension force \( f_s \) acted normal to the free surface \( n \) and depended on the surface tension coefficient \( \sigma \) and the mean curvature of the free surface \( K \):

\[
f_s = f_{s,n} \quad \text{where} \quad f_{s,n} = \sigma K n.
\] (6)

This led to artefacts: parasitic currents at the interface gave rise to velocities that were much larger than those in the rest of the fluid. They were damped using an Interface Momentum Dissipation (IMD) model, which adds artificial viscosity at the surface. Large values of IMD can have a detrimental effect on wave behavior. There is no general cut-off value for IMD; it needs to be determined on a case-by-case basis. Figure S3 shows that the addition of 1.0 IMD damping to the CFD model virtually eliminated the excessive mean and average velocities seen at the surface with lower or no damping. A value of 3.0 was chosen; it had no detrimental influence on the overall pattern of flow but improved convergence of the solution compared to a value of 1.0.

The IMD value of 3.0 was used in conjunction with a surface tension of 72 mN/m and a contact angle of 70°, which is the contact angle of water with poly(methyl methacrylate) (PMMA). The time step was reduced to \( 5 \times 10^{-5} \) to satisfy the CFL criterion. The number of mesh cells was maintained at 652,000 following a further mesh convergence study which showed that it gave mean WSS values indistinguishable from those obtained with 1,350,000 cells and maximum WSS values that were only around 10% lower than with the finer grid (Figure S4).

In additional simulations, the model with surface tension and wetting was run with the rpm of the shaker altered from 150 to 100 rpm, and with the fluid properties changed from those of water to those of ethanol (Table 1). The contact angle for ethanol is 20°. IMD values of 10 and 2 were used for the 100 rpm and ethanol models, respectively.

The instantaneous shear stress components at each mesh point on the base of the well were extracted at every 10th time step and post-processed using a custom script in MATLAB (2020a). Established shear metrics were plotted from the center to the edge of the well. For comparison with the PIV experiments, which used a laser light sheet that was comparable in thickness to the depth of liquid in the well, two-dimensional (2D) maps representing a vertical projection of velocities in the three-dimensional (3D) liquid domain were also created. Briefly, the mesh and velocity information from the simulations with and without surface tension plus wetting (CFD+ST and CFD-ST) were extracted, the liquid domain was sliced into 0.05-mm-thick layers using ParaView (v.5.4.1; www.paraview.org), and the velocity information averaged.

### 2.5 Synthetic particle image generation

Using a custom Python script, the liquid domain of the CFD model was randomly seeded with 200,000 or 500,000 particles. The particle positions were then displaced linearly according to the velocity field using ParaView. The positions of the original and displaced points were processed through the PIV Synthetic Image Generator (SIG) (Lecordier & Westerweel, 2004) to produce an image pair. The settings for the SIG were kept the same as the experimental set-up.

A range of different parameters were tested using the SIG. The thickness of the laser sheet was varied between 3.75, 7, and 10 mm, the laser sheet profile was Gaussian or uniform, and the location of the laser sheet was moved \(+1, +2, or -1 \text{ mm}\) from its original position. All of these parameters were tested while varying the time between laser pulses from 0 to 4000 µs.

### 2.6 Experimental PIV setup

Figure S5 illustrates the PIV apparatus. An optical dovetail rail (Thorlabs) was screwed onto the platform of the orbital shaker. It was used to secure a high-speed camera (Dantec Dynamics FlowSense EO) and a custom-made PMMA well sitting on a 3D printed frame that held a mirror at 45° to its base. The PMMA well had the same dimensions as one well of a Corning Costar® 12-well plate. A 532-nm laser (Laser Nano L laser head with an LPU550 PSU power head), synchronized with the camera and having a pulse interval of 1 ms, was placed 1 m away. A silica plano-concave cylindrical lens with a focal distance of 500 mm and a silica plano-convex lens with a focal distance of \(-25 \text{ mm}\) were used to produce a flat laser sheet parallel to the base of the well.

To overcome reflections, the back inner wall of the well was sprayed with red-orange matt fluorescent paint (Rust-Oleum) which fluoresced at \(\geq600 \text{ nm}\). A \(532 \pm 2 \text{ nm}\) band-pass filter was placed in front of the camera.

### Table 1 Summary of the different models and corresponding dimensionless numbers

| Fluid | rpm | \(v\) mm²/s | \(\rho\) kg/m³ | \(\sigma\) mN/m | IMD | Re | F | Bo | We | \(\Delta h/2R\) |
|-------|-----|-------------|-------------|---------------|-----|----|----|----|----|-----------|
| Water | 150 | 0.78        | 1000        | 72.0          | 3   | 2466 | 0.13 | 69 | 9 | 0.18 |
| Ethanol | 150 | 1.1         | 785         | 21.8          | 2   | 1369 | 0.13 | 172 | 24 | ND |
| 100 rpm | H₂O | 100 | 0.78 | 1000 | 72.0 | 10 | 1644 | 0.06 | 69 | 4 | 0.07 |

Note: \(v\), kinematic viscosity; \(\rho\), density; \(\sigma\), surface tension; IMD, interface momentum dissipation value; Re, Reynolds number; F = \(\alpha w^2/g\) is the dimensionless forcing; Bo, Bond number; We, Weber number; \(\Delta h/2R\) is the dimensionless criterion (Alpresa et al., 2018a) that determines whether wave breaking occurs.
lens to pass the 532-nm laser light scattered by the particles while rejecting the higher wavelength fluorescence from the paint.

The water in the well was seeded with polyamide particles having a mean diameter of 5 µm, diameter range 1–10 µm, and density 1003 kg/m³ (Dantec Dynamics). The hydrophilicity of the particles was increased by plasma treatment for 1 min before dispersion.

To ensure that the wave was captured in the same position in each orbit, and hence to allow averaging of images, an external trigger signal was generated using a metal strip secured to the shaker platform so that it crossed an Arduino photo interrupter module once every rotation. The maximum camera trigger rate was less than the shaker rotation frequency, so every third rotation was captured.

2.7 | PIV processing

Image pairs were post-processed using PIVlab, a Particle Image Velocimetry tool for MATLAB (Thielticke & Stamhuis, 2014). A circular mask was used to isolate the area of interest and contrast-limited adaptive histogram equalization (CLAHE) was applied to improve the image quality.

Each image was sub-divided into interrogation windows of 64 × 64 pixels (pixel size = 6 µm). Two passes were used, 64 × 64 and 32 × 32 with a step size of 50%. Interrogation windows were cross-correlated between image pairs by fast Fourier transform window deformation:

$$C(m, n) = \sum_i \sum_j A(i,j)B(i - m, j - n),$$

where i and j are the length and width of interrogation window and C is the correlation matrix giving the best displacement of particles between interrogation windows A and B. The displacement between an interrogation window in the first frame and the window from the second frame giving the highest cross-correlation with it, divided by the time delay between the images (which is equal to the time delay between laser pulses), gave a velocity for each window. Velocity outliers (magnitudes > 0.22 m/s) were replaced by interpolated vectors. One image pair was used to produce each 2D vector map for the experimental data and an average of 10 pairs for the synthetic data.

As with the CFD-ST and CFD+ST models, PIV experiments were also conducted after the rpm of the shaker was changed from 150 to 100 rpm and after the liquid was changed from water to ethanol.

2.8 | Measurement of fluid height in a swirling well

To map the height of the fluid in a swirling well, 14 mg/ml EBD was added to the liquid in the well, a light sheet was placed under the well to trans-illuminate the fluid from below and a camera (Samsung S8) was secured on the orbital shaker platform 10 mm above the center of the well (Figure S6). The camera was focused on the bottom of the well. Imaging parameters were: sensitivity, 100 ISO; aperture, f/1.7; white balance, 5400 K; exposure time, 1/180 s; image size, 4032 × 1969 pixels.

Initially, the well was stationary and images were taken with decreasing volumes of EBD solution, from 2304 to 192 µl in increments of 192 µl (corresponding to a height of 6–0.5 mm in increments of 0.5 mm, in the absence of a meniscus). Images were then taken of the swirling well with the platform translating at 100 rpm or 150 rpm, using 767 µl of EBD solution (corresponding to a mean height of 2 mm).

Images were post-processed in MATLAB R2016a. Pixel intensities from the images of the static well were inverted and fitted to a theoretical meniscus (Equation 3) calculated for surface tension 70 mN/m and contact angle 57°. An exponential was fitted to data obtained with a stationary well to produce a calibration curve of liquid height against pixel intensity. This was then used to convert pixel intensity to height for the swirled fluid. For statistical analysis, Pearson’s correlation coefficients were calculated for experimental versus CFD-ST and CFD+ST height plots.

3 | RESULTS

3.1 | Overview

Measurements of surface tension and contact angle are reported first, followed by the effects on wave breaking, fluid velocities and WSS of incorporating them into the numerical model. Results of synthetic and actual PIV are then described and compared with numerical results obtained with and without surface tension plus wetting. Finally, the experimental and numerical height maps are presented.

3.2 | Measurements of surface tension and contact angle

Surface tension data are shown in Figure S7. The value for distilled water at 37°C was 69 mN/m, as expected, and little affected by the addition of RB or EBD. Complete cell culture medium gave a substantially lower value, presumably because proteins in the FBS act as detergents, disrupting hydrogen bonds. (A value of 60 mN/m, closer to that of water, was obtained for serum-free medium). The value for ethanol was lower still.

Contact angles of 67° and 57° were obtained for solutions of RB and EBD, respectively.

3.3 | Effects of incorporating surface tension and wetting into the CFD model

3.3.1 | Wave breaking

The CFD-ST model showed a breaking wave, visible as ruffled height contours in a 3D rendering (Figure 1), that started at the edge of the well and spanned almost to the center of the well. The CFD+ST
model showed a smooth wave front with no discontinuities in the liquid-air interface (Figure 1). The 2D projections of surface height in the two models, discussed below, are consistent with this observation and with the fact that adding surface tension, and hence a meniscus, elevates the height near the edge of the well at all points around its circumference. A corollary is that the minimum surface height is no longer at the wall.

3.3.2 Velocities within the fluid

Figure 2a shows the mean and maximum magnitudes of fluid velocity within each x–y plane as a function of depth within the well. Data are shown before and after incorporating surface tension and wetting into the CFD model. Note that these plots are not velocity profiles. Note also that since the surface height of the swirling fluid varied from 0.4 to 4.2 mm depending on location in the x–y plane, the values at the top of the curve are derived from a smaller number of sample volumes than those at the bottom of the curve. Mean and maximum velocities were approximately independent of height in layers situated between 0.5 and 3 mm from the bottom of the well. They were lower in the highest layers, and declined between 0.5 mm and the base of the well, where they were forced to zero by the no-slip condition. Adding surface tension and wetting substantially decreased both mean and maximum velocity magnitudes except within the boundary layer at the base of the well.

For comparison with the PIV simulations presented below, Figure 2b shows the mean and maximum velocity magnitudes, averaged over increasing heights from the base of the well. The first horizontal slice through the fluid includes heights of 0–0.05 mm from the base of the well, the second 0–0.2 mm, and so on. For the CFD-ST model, there was little difference in the mean velocity in the lowest 1 mm, and when volumes above that height were also included. This behavior was almost unchanged when surface tension and wetting were included, reflecting the similarities in velocities below 1.5 mm (Figure 2a) and the relatively small volumes above that height. Concerning maximum values, there was a relatively constant value in the CFD-ST model once heights up to approximately 2 mm had been included, and for the same reasons. For the maxima, however, adding surface tension and wetting decreased the maximum values, and the height at which the plateau was reached. Again, this is consistent with the lower maximum velocities and the lower height at which the highest value was reached, seen in Figure 2a.

3.3.3 Shear stress at the base of the well

We next consider shear stress on the base of the well. Note that the term WSS is used, by analogy with the hemodynamic stress exerted on the inner surface of the wall of blood vessels.

There was a similar pattern of shear stress at the base of the well in the CFD-ST and CFD+ST models except at the leading edge of the wave, where there was a larger region of moderate shear and a higher maximum shear when surface tension and wetting were added (Figure 3a); the change presumably reflects the alteration in wave characteristics described above and discussed further below. WSS is the product of viscosity and the near-wall velocity gradient. Since the viscosity was not changed between the two models, the results indicate that the flow at the base of the well experienced a steeper velocity gradient with surface tension and wetting than without in the areas where WSS is greatest.

The polar plots in Figure 3b represent the magnitude and direction of instantaneous WSS vectors at the base. At a radial distance of 0.5 mm, the plots for both models are circular, showing that the
vector swept around the well with constant velocity, but the magnitudes in the surface tension plus wetting model were slightly higher.

Progressing towards the edge of the well, the difference in pattern between the two models increased. By 10.5 mm from the center, the polar plot for the CFD-ST model is a narrow ellipse. Where the points lie close to the vertical centreline in the bottom half of the plot, the instantaneous vectors were parallel to the edge of the well and pointing in the predominant direction of wave travel. Over the rest of the cycle, where the points are confined to the lower left quadrant, the vectors additionally had components pointing towards the center of the well. In the model with surface tension and wetting, WSS vectors extend much further into the top half of the plot and less to the left in the bottom quadrant. The confinement to regions that are close to the centreline in both upper and lower quadrants implies nearly uniaxial flow over all the cycle (i.e., flow that was always circumferential but reversed direction). Points are sparser in the upper quadrant, meaning the vector was oriented away from the predominant direction of wave travel for only a small fraction of the cycle. However, the magnitudes during this brief excursion were up to three times greater than those seen when the vectors pointed in the direction of wave travel. Thus the surface tension plus wetting model experienced higher WSS magnitude, and more strongly uniaxial flow with a greater reverse component.

3.3.4 | WSS metrics

WSS metrics are plotted radially from the center to the edge of the well in Figure 3c, again using data from the models with and without surface tension plus wetting.

The time average WSS (TAWSS) is an average over one cycle of the magnitudes of the instantaneous vectors. In the control model, it had an approximately constant value from the center to a radial distance of 7.5 mm; it then increased, and there was a decrease at the edge of the well due to the no slip condition at the wall. The bump in the curve produced by these opposing factors is consistent with the polar plots in Figure 3b, which show instantaneous WSS vectors extending further from the 0,0 origin with increasing radial distance from the center of the well. When surface tension and wetting were added, however, the values up to 7.5 mm were increased, and values beyond that were decreased, leading to a much smaller overall dependency on radial position and a reduced bump in the curve.

The Oscillatory Shear Index (OSI) captures any vectors that are not aligned with the mean vector. In the CFD-ST model, it decreased from the center, where the flow is highly multidirectional, to the edge, where the flow is more nearly unidirectional, the rate of decrease being steeper at radial distances beyond 7 mm. Again, the boundary condition at the wall reversed this trend. Adding surface tension and wetting eliminated the steepening of the gradient at 7 mm, and thus reduced the dependence on radial position.

Transverse WSS (transWSS) captures components of the instantaneous vectors that are at right angles to the mean vector. Thus, unlike the OSI, it does not include vectors that are at 180° to the mean vector, but only those that diverge from the mean axis. In the CFD-ST model, transWSS was approximately constant from 0 to 6 mm, and then decreased gently until near the edge of the well, where there was a steeper decrease; presumably the increase in oscillatory but uniaxial flow failed to compensate for the decrease in off-axis flow beyond this point. When surface tension and wetting were included, transWSS was higher in the plateau region and then decreased more sharply at higher radial distances. Thus, unlike
TAWSS and the OSI, radial changes in transWSS were increased in the CFD+ST model. The Cross Flow Index (CFI) is similar to transWSS but does not take account of vector magnitudes; only the direction of the instantaneous vectors is considered. Like transWSS, it decreased from a radial distance of 6 mm onwards. Unlike transWSS, it was unaffected by incorporating surface tension and wetting. This suggests that the effect of surface tension plus wetting on transWSS were caused by changes in vector magnitude rather than vector direction.

The magnitude of the mean WSS vector (MagMeanWSS) was zero at the center of the well, where the nearly perfect multidirectionality meant that the vector in each direction was canceled by one at 180° to it. It rose slowly to a radial distance of 7.5 mm, and then more rapidly to a radial distance of 10.5 mm. Polar plots representing the magnitude and direction of instantaneous WSS vectors through one orbit. Each panel corresponds to a different distance along a radius from the center to the edge of the well. Each line in the plot (one for CFD-ST and one for CFD+ST) is the locus of points representing the position of the tip of a WSS vector with its origin at 0, 0; note that this is the local origin and not the center of the well. Points on the line are spaced at equal time increments. The Cross Flow Index (CFI) is a measure of the oscillatory shear index. It is calculated from the mean of the WSS vector and is unaffected by the addition of surface tension and wetting.
then increased rapidly until decreasing at the edge of the well, leading to a bump like that seen in the TAWSS curve, and reflecting the same causes. Adding surface tension and wetting reduced the increase after 7.5 mm. Hence like the TAWSS and the OSI, its dependence on radial location was reduced in the modified model.

The transWSSmin and CFImin are generalized version of the transWSS and CFI that described the values of the latter two metrics if they are calculated relative to the angle that minimizes them, rather than relative to the orientation of the mean WSS vector. Neither of the minimized metrics was substantially affected by modifying the model.

Figure 3d shows the direction of the mean and modal WSS vector. Angles of +90° and −90° correspond to vectors that are parallel to the edge of the well, with −90° being in the predominant direction of wave motion and +90° being the opposite direction. The broad trends were unaffected by modifying the model, but local spatial fluctuation in the modal WSS direction was damped by the addition of surface tension. Figure 3d also gives the reference orientations associated with the transWSSmin and CFImin, and these are substantially changed in the modified model.

### 3.4 Synthetic PIV

Velocities obtained from idealized simulations of PIV, based on computed flows, were compared with the computed flows themselves to optimize the parameter values for PIV, to validate the PIV approach in the absence of experimental errors, and to compare models with and without surface tension plus wetting.

Figure 4 shows effects on average and maximum velocities in the synthetic PIV solution of varying the vertical location of the laser sheet, switching from a Gaussian to a uniform beam profile, changing the interval between laser pulses and increasing the number of particles seeded. All simulations were based on the CFD-ST flow model. The average and maximum velocity obtained directly from the CFD-ST solution (0.19 and 0.063 m/s, respectively) are shown for comparison.

An increase from 200,000 to 500,000 particles produced negligible differences in the average velocity but dramatically reduced the large errors in maximum velocity seen at the lower seeding density for pulse intervals below 1000 µs.

Changing the light sheet position and profile had relatively small effects; in general, the highest errors were seen at z = +1 mm. (This
position refers to the bottom of the light sheet). Switching to a thicker sheet (7 instead of 3.75 mm) had a negligible effect. Using a uniform rather than a Gaussian beam profile did have an effect, but only for the maximum velocity when detected with the high seeding density and a delay of around 1000 µs, where it increased the discrepancy with the CFD result. Thus a laser sheet with Gaussian profile and thickness of 3.75 mm appears sufficient despite the maximum height of the liquid reaching 4.2 mm in the computational models, and the results are insensitive to the precise position of the sheet so long as the first millimeter above the base of the well is included.

The interval between pulses had by far the largest effect. The average velocity was highest at 10 µs but dropped by 100 µs, beyond which it remained fairly constant and close to the CFD value, until 1000 µs; it continued to drop as the time between laser pulses was increased further. The maximum velocity was also closest to the maximum CFD velocity at ≈1000 µs but increased sharply prior to 1000 µs as well as for longer delays.

Thus 1000 µs seem to be the optimum interval for accurately capturing the flow fields. At this delay, there was negligible effect of seeding density on either the average or the maximum velocity and, as already noted, the height of the laser sheet also had only minor effects with the exception of position $z = +1$ mm.

Regardless of the number of particles and the position or profile of the laser sheet, the maximum velocity at 1000 µs was always slightly higher than the result obtained directly from the CFD model. For example, at 500,000 particles, with the laser sheet in its original position, the maximum synthetic PIV velocity was 0.21 m/s, whereas the maximum CFD velocity was 0.19 m/s.

Figure 5 shows the CFD solution and the corresponding synthetic PIV simulations. The laser sheet was 3.75 mm thick and positioned parallel to the base of the well with the center of its Gaussian profile 1.875 mm above the base. The number of seeding particles was 500,000 and the time delay was 1000 µs. The velocity direction, velocity magnitude and streamlines are shown. CFD results are averaged over the full fluid height to correspond to the PIV simulations, obtained with a thick laser sheet.

The CFD and the synthetic PIV solutions agree well with each other in both models. However, there is a substantial effect of adding surface tension and wetting: the magnitudes are lower (note the different color bar scale), there is a different pattern of vector direction and magnitude at the front of the wave, consistent with the altered pattern of WSS described above, and the positions of the source and sink of the streamlines are changed.

### 3.5 Experimental PIV

Streamlines and 2D maps of flow vectors derived from experimental PIV are shown in Figure 5c, adjacent to the corresponding CFD and synthetic PIV results. Since the working fluid was water, the maps should be compared with those for the CFD and synthetic PIV models that incorporated surface tension and wetting (Figure 5b). The same general features are seen but there are a number of discrepancies: the overall magnitudes are lower, the arc of rapid flow that lies next to the edge of the well at the top of the map in the simulations is lower in magnitude and displaced towards the center, and the source for the streamlines is located further from the edge and closer to the sink. Nevertheless, the peak of high velocity at the left-hand side of the plot is correctly captured (and is missing from the CFD-ST simulation and corresponding synthetic PIV plot).

![Figure 5](image_url)
CFD-ST, CFD+ST and experimental PIV velocity maps obtained using ethanol as the working fluid or with rotation at 100 instead of 150 rpm are shown in Figure 6. Ethanol was used to reduce surface tension (and is also likely to have affected particle dispersion) and 100 rpm was used to eliminate the breaking wave even in the CFD-ST model. Under both conditions, there appeared to be a better agreement between the experimental PIV and the CFD+ST model than was observed for the baseline (150 rpm, water) case shown in Figure 5. (Note that there is a readily apparent discrepancy between CFD-ST and CFD+ST simulations for ethanol in Figure 6, as there was for the baseline case shown in Figure 5).

The average and maximum velocity magnitudes calculated for each map are given in Table 2. In the baseline case, values for experimental PIV are closer to the CFD+ST model than was observed for the baseline (150 rpm, water) case shown in Figure 5. Nevertheless, the discrepancies between CFD+ST and PIV are substantial and most likely reflect the presence of the arc of rapid flow that lies next to the edge of the well at the top of the map in the former but not the latter. With ethanol, there is better agreement of experimental PIV with CFD-ST, whereas in the 100 rpm case, which agrees better depends on whether the average or maximum velocity is considered. In both the latter cases, this failure to obtain best agreement with the CFD+ST map appears to reflect the systematic difference in overall magnitudes between the maps; as already noted, the pattern of velocities seen with experimental PIV is clearly closest to that simulated with surface tension and wetting (Figure 6). In neither case is the disagreement as large as for the baseline model, presumably reflecting the fact that the arc of rapid flow at the top of the well is seen in both CFD+ST and PIV in the ethanol case, and in neither for the 100 rpm case.

### Table 2

|                | Average velocity (m/s) | Maximum velocity (m/s) |
|----------------|------------------------|------------------------|
| **Baseline**   |                        |                        |
| CFD-ST         | 0.0576                 | 0.194                  |
| CFD+ST         | 0.0493                 | 0.128                  |
| PIV            | 0.0212                 | 0.0523                 |
| **Ethanol**    |                        |                        |
| CFD-ST         | 0.0520                 | 0.184                  |
| CFD+ST         | 0.0439                 | 0.105                  |
| PIV            | 0.0504                 | 0.1958                 |
| **100 rpm**    |                        |                        |
| CFD-ST         | 0.0120                 | 0.0205                 |
| CFD+ST         | 0.0098                 | 0.0177                 |
| PIV            | 0.0083                 | 0.0291                 |

Abbreviations: CFD, computational fluid dynamics; PIV, particle image velocimetry; ST, surface tension plus wetting.
The experimental PIV maps were rotated by 0°–360° and the correlation coefficient with the corresponding CFD models was calculated. Separate Pearson’s coefficients were calculated for the velocity magnitude and the vector orientation since the two gave best matches at different rotations; the average of the two correlation coefficients is shown in Table 3. For the baseline (150 rpm, water) and ethanol simulations, experimental PIV correlated better with the CFD+ST than the CFD-ST model. (The coefficients were approximately 50% higher). Both correlation coefficients for the ethanol simulations were higher than both coefficients for the baseline simulations. Experimental PIV correlated better with the CFD-ST than the CFD+ST model in the 100 rpm simulations; nevertheless, both coefficients were still higher than both coefficients for the baseline case.

### TABLE 3  Correlation coefficient for the CFD-ST or CFD+ST map with the PIV map for the baseline, ethanol and 100 rpm cases

|            | Average r value |
|------------|-----------------|
| Baseline   |                 |
| CFD-ST vs. PIV | 0.35           |
| CFD+ST vs. PIV | 0.52           |
| Ethanol    |                 |
| CFD-ST vs. PIV | 0.62           |
| CFD+ST vs. PIV | 0.89           |
| 100 rpm    |                 |
| CFD-ST vs. PIV | 0.74           |
| CFD+ST vs. PIV | 0.63           |

Note: The maps were rotated to achieve the best correlation coefficient for either the vector magnitude or the vector direction, and the average of these two r values is tabulated.

Abbreviations: CFD, computational fluid dynamics; PIV, particle image velocimetry; ST, surface tension plus wetting.

### 3.6 | Experimental versus computational height maps

The calibration curve for the relation between liquid height versus pixel intensity, obtained by microscopy of static wells containing various volumes of a dye solution, is shown in Figure S8, together with the exponential curve fitted to the data. The exponential was truncated at 10.4 mm from the edge of the well to avoid errors caused by residual reflections from the side wall.

The calibration was then applied to obtain the height of the liquid across wells that were swirled at 150 or 100 rpm, and the results were compared to corresponding CFD-ST and CFD+ST height maps (Figure 7). To match the phases between computed and experimental height maps, the experimental height maps were rotated as above.

![Figure 7](image-url)

**Figure 7**  (a) Height of the free surface obtained by experiment and by CFD simulations with and without surface tension plus wetting (CFD+ST, CFD-ST), and corresponding pixel-by-pixel scatter plots and their correlation coefficients for (a) baseline and (b) 100 rpm cases. The black circles are drawn at a radial distance of 10.4 mm from the center of the well; experimental data beyond that are less accurate due to optical reflections from the side wall. Note that wave breaking, evident as a disturbed leading edge to the wave, is only apparent in the baseline model. CFD, computational fluid dynamics; PIV, particle image velocimetry.
and the rotation that gave the highest Pearson’s correlation coefficient between the two was used.

At both rotational speeds, the experimental data agreed better with the CFD+ST height map than the CFD-ST one. (The correlation coefficients are also shown in Figure 7). The correlation coefficients were lower at 100 than 150 rpm. The overall correlation coefficients at 150 rpm were >0.9 with or without surface tension plus wetting. Consistent with the velocity maps, the maps from CFD-ST simulations showed less curvature than the CFD+ST maps or the experimental maps, and the presence of surface tension, either in the model or in the experiment, was accompanied by the minimum height being displaced from the edge of the well.

Only the CFD-ST map for 150 rpm showed wave breaking. To investigate this further, the 150 rpm maps were split into four quadrants (Figure 8). The majority of the breaking wave in the CFD-ST map and the lowest height values are located in quadrant 1. Pearson’s correlation coefficient was used to compare the model and experimental results independently for the four quadrants. Quadrant 1 had a substantially lower coefficient than the other quadrants when comparing the experimental and CFD-ST or experimental and CFD+ST height plots, and the reduction in r value was greater when surface tension and wetting were neglected (Figure 8b,c). (Overall, there was a substantially better agreement of experimental values with the CFD+ST heights than with the CFD heights). This result is consistent with the discrepancy between experimental and CFD-ST maps being caused both by low heights and wave breaking.

4 | DISCUSSION

Numerous studies have used the swirling well system to examine effects of different shear stress profiles on endothelial cells but fewer have used CFD to characterize the flow and only rarely has the CFD incorporated surface tension or the flow been examined by experimental methods. In the present study, we simulated flow using CFD and used experimental methods—PIV and optical height measurement—to assess the accuracy of the CFD and to determine whether it was necessary to incorporate surface tension and wetting into the numerical model. Simulations of the PIV were also conducted to optimize the experimental parameters and to examine whether the experimental technique as implemented would be expected to give an accurate characterization of the flow even in the absence of experimental error. We also investigated the effects of switching to a fluid—ethanol—with a lower surface tension than water, to increase Bo and We, and of using a reduced linear velocity, to decrease We.

In brief, we found that fundamental features of the flow simulations were altered by incorporating surface tension and wetting, that synthetic PIV characterized the flow accurately even though the thickness of the laser sheet precluded capturing flow in the vertical plane, and that experimental PIV agreed well with the numerical results so long as surface tension and wetting are included.

The study was motivated by a discrepancy between computational models and experimental observations. Alpresa et al. (2018a)
numerically simulated a wide range of swirling well configurations and found that the wave would break if:

$$\Delta h/2R \geq 0.7\Gamma,$$  \hspace{1cm} (8)

where $\Delta h$ is the free-surface amplitude, $R$ the well radius, and $\Gamma = d/R$, where $d$ is the average fluid height. For the parameters used in our previous studies, $\Delta h/(2R)$ was >0.7 and wave breaking was therefore expected. However, a subsequent slow-motion video of flow within the well did not show such behavior: the wave had a smooth surface. Importantly, surface tension was neglected in the CFD model that was used by Alpresa et al. to define this criterion.

Two previous studies have compared flow fields obtained by CFD and by optical methods. Neither incorporated surface tension or wetting in the CFD model and therefore neither is able to resolve the issue we investigated. A study by Salek et al. (2012) used PIV to obtain velocity fields in one well of a six-well plate. There was good agreement between CFD and PIV. (Due to reflections from the side walls, however, it was not possible to resolve vectors within 5 mm of the edge of the well). The CFD-derived height maps showed wave breaking in two of four cases (their fig. 11b and d), as predicted by the criterion given in Equation (8) and by the modified equation given at the end of the Conclusions section, below. A more recent study carried out by Thomas et al. (2017) compared CFD data with PIV measurements that spanned the entire diameter of the well. Velocity vector components over one cycle differed <5% between CFD and PIV, and magnitudes averaged over the inner 20% of the radius differed by only 2.4%. However, the mean fluid depth in the experimental study was 10 mm, which is fivefold greater than in our study; Equation (8) suggests that increased mean fluid height reduces the likelihood of wave breaking.

In our study, wave breaking was clearly visible in the height maps produced by a conventional CFD model and was eliminated when surface tension and wetting were incorporated. The modified model also gave greater height near the edge of the well and moved the minimum height away from the wall, and mean and maximum velocities within the fluid were reduced except in the boundary layer at the base of the well.

Modifying the model had a major impact on WSS at the base of the well. Its magnitude was reduced and its pattern at the wavefront was altered. There was negligible difference in the plot of mean WSS vector direction versus radial distance, but differences were seen in the instantaneous vectors: flow was purely multidirectional close to the center but had greater magnitude when surface tension and wetting were included, while towards the edge the modified model gave more uniaxial behavior and greater reverse flow. There were corresponding changes in the dependence of various WSS metrics on radial location. The relative changes in TAWSS and transWSS are of particular significance. If the well is divided into edge and center regions having equal area, which can be achieved by using a radial distance of 7.8 mm as the boundary, then the difference in TAWSS between the two regions is reduced from being 30% lower in the center to being only 7% lower, whereas transWSS is hardly changed (58% higher at the center without surface tension and wetting, and 53% with them). Differences in cell behavior between the two regions observed in previous studies are thus more likely to reflect changes in multidirectionality than in shear magnitude.

Before testing the CFD results against experimental PIV, we first optimized the parameters to be used in the PIV experiments by using “synthetic PIV,” a computational model of PIV generated by applying the synthetic image generating software of Lecordier and Westerweel (2004) to our CFD simulations. Comparing the mean and maximum velocities obtained by synthetic PIV with those obtained directly from the underlying CFD showed that the critical variable was the interval between laser pulses. When that was 1 ms, agreement was good and relatively insensitive to particle density or the vertical position, thickness, and profile of the laser sheet. (Failing to capture flow near the base of the well was the next most critical issue).

Using optimum values of these parameters, the synthetic PIV and the CFD simulations from which they were generated gave essentially identical results, both when surface tension and wetting were incorporated and when they were not. Even with velocity changing almost fivefold across the well, the postprocessing algorithm was able to compute accurate flow fields using a constant interrogation window size and time between laser pulses. The maps of velocity vectors, averaged through the thickness of the laser sheet, were also consistent with the WSS maps described above and, like them, showed substantial differences between the models with and without surface tension plus wetting: the magnitudes were lower in the modified model, there was a different pattern of vector direction and magnitude at the front of the wave, and the positions of the source and sink of the streamlines were changed.

The good agreement between data obtained by synthetic PIV and directly from the underlying CFD leads to the expectation that experimental PIV should provide a true record of the real flow in the swirling well, subject only to experimental errors. A key finding was that the agreement between experimental PIV and CFD was substantially better when surface tension and wetting were added to the baseline model. Nevertheless, discrepancies remained: the experiments gave lower overall velocity magnitudes, there was a shift of an arc of high magnitudes away from the edge of the well, and the source of streamlines was further from the edge of the well.

When ethanol was used instead of water, the correlation coefficients were nearly doubled. The improved correlation may indicate an error in how the CFD handles surface tension, since ethanol has a lower value than water, which was used in the baseline configuration. However, the correlation remained higher for the model with surface tension plus wetting than for the model without them. An alternative explanation is that the seeded particles did not mix well with water despite plasma treatment, leading to clumping; this was occasionally visible by eye. Particles would disperse better in ethanol. Larger particles would hinder flow tracking (Melling, 1997) and could also lead to alteration of the flow field.

When the rotational speed of the orbital shaker was reduced from 150 to 100 rpm, the map of velocity vectors was markedly
altered, and indicated different wave behavior. Adding surface tension and wetting had little effect on the CFD results. Contrary to the other two cases, the correlation with experimental PIV was better for the CFD model without surface tension and wetting. However, the difference was small (the ratio of the highest to the lowest correlation coefficient was 1.17, whereas it was 1.49 and 1.44 in the other two cases), so this apparent discrepancy may be spurious, indicating only that surface tension is less important for this type of wave. The slower rotational rate might give poorer particle dispersion than the faster one.

Experimental validation of the CFD models also employed a second method: comparison of the measured and computed height of the fluid. The results, obtained for water at both 150 and 100 rpm, unequivocally showed excellent agreement between experiment and the computational model that incorporated surface tension and wetting, and poor agreement between experiment and the CFD-ST model. In the latter simulations, the wave shape was fundamentally incorrect at both rotational rates and the 150 rpm case additionally showed wave breaking, which was absent when surface tension plus wetting were incorporated and was not seen in the experimental results. The excellent agreement is consistent with the remaining discrepancies between the experimental PIV results and the corresponding CFD+ST models being caused by experimental rather than computational errors.

Although the majority of studies using CFD to model flow in the swirling well have not included surface tension and wetting, three have done so, and may be of value to researchers using the same experimental parameter values. Simulations by Dong et al. (2017) considered well diameters from 20 to 50 mm in increments of 10 mm, rotational rates between 60 and 300 rpm in increments of 60 rpm, and orbital radii of 5, 15, and 25 mm. Surface heights are given only for one low-speed case and wave breaking is not discussed, but WSS vectors at the base of a well are given for a range of cases. Pouran et al. (2012) examined a well with 20 mm diameter, a rotational rate of 100 rpm and an orbital radius of 6 mm; these values are reasonably close to those studied here. The solutions showed “splashing” for certain fluid heights, but the simulations appear only to consider the first quarter of an orbit after starting the shaker, when it is unlikely that a steady-state has been reached. A numerical model was developed by Toro et al. (2018), based on equations from Lubarda (2013), to derive free surface profiles, and was applied to 96-well and 384-well microplates on an orbital shaker rotating at rates >500 rpm. When surface tension was included, there was good agreement with surface profiles obtained by imaging over a wide range of parameter values. There was poor agreement in the absence of surface tension. Furthermore, numerical solutions for dimethyl sulfoxide, which has a surface tension of 48 mN/m, gave surface profiles that were markedly different to those for water (72 mN/m), again suggesting that surface tension has a large effect.

Toro et al. (2018) also modeled two contact angles—87.4° and 102.1°—to represent water in, respectively, polystyrene and polypropylene wells. This had a much smaller influence on surface profiles: for example, surface tension had a >20-fold larger influence on maximum wave height (>200% change vs. <10% change). Surface tension acts to minimize the free surface area of a liquid and, for swirling liquid in a cylindrical container on an orbital shaker, will therefore act to flatten the surface. The contact angle governs the profile at the point where the free surface intersects the side wall. Changes in contact angle can influence the profile further in, but are unlikely to have the same dampening effect on wave behavior.

The present study suffered from a number of limitations:

1. Experimental height measurements agreed better than experimental PIV data with the results of CFD+ST simulations. This is consistent with the presence of experimental errors in the PIV. We have discussed above the possibility that the seeded particles clumped together. Other potential sources of error are the thickness of the laser sheet and the failure to capture velocities in the z-plane, although these limitations were also present in the synthetic PIV, which agreed well with the numerical results.

2. A constant contact angle was assumed, whereas in practice the contact angle could change between advancing and receding parts of the wave. However, the work of Toro et al. (2018) suggests that changing the contact angle has only minor effects even in much smaller wells.

3. The baseline simulations were conducted using water as the working fluid; full cell culture medium had a lower surface tension (47 mN/m) than water (72 mN/m) at 37°C. PIV was not attempted with full cell culture medium as it is prone to bubble formation. In preliminary numerical studies, however, lowering the surface tension to this value resulted in negligible changes to the radial distribution of WSS metrics, the orientations of the mean WSS vector, and the directions associated with transWSSmin and CFimin (Figure 59). The correlation coefficient was 0.999 between simulations with surface tension values of 47 and 72 mN/m. Thus, unlike the cases examined by Toro et al. (2018), there appears to be a threshold value of surface tension, below 48 mN/m, above which wave breaking is prevented and the dynamics are altered.

4. Surface tension and contact angle were not changed individually and hence their effects cannot be separated. However, as noted above, Toro et al. (2018) showed that influences of surface tension on the surface profile were >20-fold larger, and we would expect that discrepancy to be even greater in the present study because it used bigger wells in which less of the fluid behavior would be affected by the walls. In particular, the reduction in wave breaking that we observed over large portions of the well diameter in the modified model is plausibly explained by the dampening effect of surface tension.

5 | CONCLUSIONS

Surface tension and wetting should be included in simulations, despite the >10-fold increase in computational cost (>10 days vs. <1 day on the Imperial College HPC system), unless they are already known to have only negligible effects on the parameter
values of interest. The Weber and Bond numbers do not appear to be a reliable indicator of when this is likely to be the case.

The baseline parameter values used in the present study have previously been employed in earlier investigations of cell behavior, for which WSS was computed using models that did not incorporate surface tension and wetting. The different results obtained here with the modified model alter the conclusions of those studies. In particular, they increase the likelihood that radial variation in cell behavior reflects changes in transWSS rather than TAWSS.

Arpresa et al. (2018a) derived the criterion that wave breaking would occur if \( \Delta h/(2R) > 0.7\Gamma \) (Equation 8). The result was obtained from simulations of a large number of cases, but all the simulations neglected surface tension and wetting. Data from the present study suggest that the threshold value needs to be increased; breaking was not seen even at \( \Delta h/(2R) = \Gamma \).

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION
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