MICROBUBBLE VOID IMAGING: A NON-INVASIVE TECHNIQUE FOR FLOW VISUALISATION AND QUANTIFICATION OF MIXING IN LARGE VESSELS USING PLANE WAVE ULTRASOUND AND CONTROLLED MICROBUBBLE CONTRAST AGENT DESTRUCTION

CHEE HAU LEOW,* FRANCESCO IORI,† RICHARD CORBETT,‡ NEILL DUNCAN,‡ COLIN CARO,* PETER VINCENT,† and MENG-XING TANG*

*Department of Bioengineering, Imperial College London, London, UK; †Department of Aeronautics, Imperial College London, London, UK; and ‡Imperial College Renal and Transplant Centre, Imperial College Healthcare NHS Trust, London, UK

Abstract—There is increasing recognition of the influence of the flow field on the physiology of blood vessels and their development of pathology. Preliminary work is reported on a novel non-invasive technique, microbubble void imaging, which is based on ultrasound and controlled destruction of microbubble contrast agents, permitting flow visualisation and quantification of flow-induced mixing in large vessels. The generation of microbubble voids can be controlled both spatially and temporally using ultrasound parameters within the safety limits. Three different model vessel geometries—straight, planar-curved and helical—with known effects on the flow field and mixing were chosen to evaluate the technique. A high-frame-rate ultrasound system with plane wave transmission was used to acquire the contrast-enhanced ultrasound images, and an entropy measure was calculated to quantify mixing. The experimental results were cross-compared between the different geometries and with computational fluid dynamics. The results indicated that the technique is able to quantify the degree of mixing within the different configurations, with a helical geometry generating the greatest mixing, and a straight geometry, the lowest. There is a high level of concordance between the computational fluid dynamics and experimental results. The technique could also serve as a flow visualisation tool. (E-mail: mengxing.tang@imperial.ac.uk) © 2015 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Key Words: Contrast-enhanced ultrasound, Microbubble contrast agents, Mixing, Flow indicator, Microbubble void imaging, High-frame-rate plane wave imaging.

INTRODUCTION

Cardiovascular disease is strongly correlated with blood flow dynamics. There is increasing recognition of the influence of the flow field on the normal functioning of vessels and their development of pathology (Caro and Schroter 1969; Cecchi et al. 2011; Davies 2008; Friedman et al. 1983; Ku and Giddens 1983). Moreover, the flow field can be expected to play a role in the management of vascular pathology (Carlier 2003; Caro et al. 2013).

Several groups have characterised arterial geometry and attempted to infer its influence on the flow (Caro 2009; Caro et al. 1998, 2006; Davies 1995; Friedman and Ding 1997). Non-planar curvature and in-plane swirling and intraluminal mixing appear commonly present in normal arteries (Caro et al. 1996) and may be of importance at interventions, including bypass grafts and arterial stents (Caro et al. 2005, 2013; Coppola and Caro 2009; How et al. 2006; Shinke et al. 2008). However, readily applicable methods have been lacking for imaging the flow and quantifying mixing. A novel method based on controlled destruction of ultrasound microbubbles is proposed here: void imaging.

Existing imaging techniques can be used for flow visualisation in vivo. Such techniques, including magnetic resonance imaging (MRI), computed tomography (CT) and digital subtraction angiography (DSA), typically require local injection of a contrast agent or indicator, which is subsequently tracked over space and...
time. Although these techniques can provide high anatomic and geometric resolution, they still have limitations in terms of rapid and contemporaneous assessment of 3-D geometry and flow. The injection site must be upstream of the vessel of interest. The procedure can be highly invasive, and the injection of contrast medium can be difficult to control, precluding quantitative assessment of mixing. Furthermore, there is local disturbance of the flow field arising from the injection, and the physical properties of the contrast agent may render it an unfaithful flow indicator. In addition, CT and DSA involve the use of ionising radiation. Although some MRI techniques such as arterial spin labelling can track flow without contrast injection, they suffer from poor signal-to-noise ratio (Petersen et al. 2006) and currently are used predominantly to assess tissue perfusion.

An alternative approach is to image vascular geometries using modalities that are then combined with computational fluid dynamics to achieve flow visualisation and quantification (Hoskins 2008). This approach depends highly on the fidelity of the geometry obtained from the imaging data and also on the accuracy of boundary conditions.

Various ultrasound imaging techniques, including B-flow imaging (Chiao et al. 2000; Lovstakken et al. 2006), can provide both the vascular geometry and flow visualisation (Hoskins and Wells 2010). However, the signal-to-noise ratio of B-flow imaging is limited by the weak ultrasound scattering of blood cells and can become even less reliable in imaging deeper structures, where a lower ultrasound frequency is required.

Contrast-enhanced ultrasound (CEUS) with microbubble contrast agents, consisting of a gas core encapsulated within a lipid or albumin shell and typically between 1 and 7 μm, has been widely used in clinical applications for improved imaging of flow and assessment of perfusion (Cosgrove and Lassau 2010; Lindner 2009; Sboros et al. 2010). They have also shown great potential for molecular imaging and therapy (Ferrara et al. 2007; Stride and Coussios 2010), including enhancement of mass transport across endothelium. Under low acoustic pressure, microbubble contrast agents are able to significantly enhance ultrasound signal from within blood vessels without disturbing the flow. This has been combined with particle/speckle-tracking algorithms (known as ultrasound imaging velocimetry or echo-particle image velocimetry) to quantitatively map the flow field in vessels that light cannot penetrate (Poelma et al. 2012; Zhou et al. 2013).

Although the aforementioned techniques are capable of visualising flow and quantifying the velocity-derived parameters, experimental quantification of intraluminal mixing in vivo has not yet been reported; existing studies of mixing have mainly been undertaken using numerical models (Cookson et al. 2009). Nevertheless, quantification of intra-luminal mixing remains desirable given its relationship to conduit geometry, the flow field (including its stability and pulsatility), wall shear and fluid-wall mass transport (Caro et al. 2013; Coppola and Caro 2009; Tarbell 2003).

Secondary motion can markedly influence intraluminal mixing in larger vessels and their normal or disturbed physiology. It is important, therefore, to distinguish the contributions of advection (bulk flow) and diffusion. In determining the contribution of advection, it is desirable that a contrast agent should have a low diffusivity, such that any mixing results predominantly from advection. This situation is represented by a high Peclet number, Pe (a dimensionless quantity representing the relative rates of advection and diffusion).

Microbubbles, because of their micrometre scale relative to water molecules (nanometre scale) will have very low diffusivity in water and blood. In dilute suspension, as in this work, and from the Stokes–Einstein law, a typical bubble with a radius of 3.0 × 10^{-6} m has a diffusion coefficient $D = 7.6 × 10^{-14}$ m²/s and a Peclet number $Pe = 3.5 × 10^3$ at physiologic flow rates and scales involved in this study. Consequently, any mixing will predominantly be the result of advection rather than diffusion, and any further discussion of mixing in relation to microbubbles will relate to advective mixing. The low diffusivity of microbubbles renders them highly suited for use as contrast agents for assessing advective mixing.

A unique property of microbubble contrast agents is that at higher acoustic pressures, they can be disrupted in a highly controlled way both spatially and temporally; that is, bubbles can be “switched off” at will. In clinical practice, this property is widely employed in the so-called “destruction–replenishment” mode to quantify tissue perfusion (Tang et al. 2011b; Wei et al. 1998) using ultrasound amplitudes within clinical safety limits. This offers the opportunity to “inject” a volume void of microbubbles non-invasively, and essentially instantaneously, by increasing ultrasound amplitude in the region of interest and observing the evolution of such a bubble void in vascular space over time.

In this study, we investigated a novel method for visualising flow and quantifying mixing in large vessels, making use of controlled microbubble destruction and high-frame-rate ultrasound. An in vitro experiment was performed on three different vascular geometries, and results were compared with numerical computational fluid dynamics (CFD) solutions.

**METHODS**

**Experimental flow setup**

Vessel-mimicking phantoms of three geometries—straight, planar-curved and helical—were constructed
from rubber latex tubing (Primeline Industries, Akron, OH, USA) with an internal diameter ($D_i$) of 0.006 m. Figure 1 illustrates diagrammatically the three vessel geometries. Location $A$ in Figure 1 refers to the location of a single-element transducer $3.5 \times 10^{-2}$ m in diameter used for microbubble destruction, that is, the location of the void indicator “injection”; 0.2 m downstream is the imaging plane (location $B$), with the respective location markers representing the central lines of the two transducers.

All phantoms were submerged (approximately 0.03 m) in a water tank filled with degassed water. The surfaces of the transducers were submerged (approximately 0.01 m) in the water bath directly above the tubing at locations $A$ and $B$. The straight phantom (Fig. 1a), consisted of a straight tube 0.4 m long with an entrance section of length 0.09 m ($L_{E}$) to ensure established Poiseuille flow (Caro et al. 2012). Void was created in the tubing directly underneath the upstream transducer ($A$), and after the distance travelled by the void ($AB$), images were acquired with the detection transducer downstream ($B$). The curved phantom (Fig. 1b) consisted of a straight entrance section of length 0.09 m, before the flow entered a planar curve of arc length $\pi$ rad, with a radius ($R_c$) of 0.16 m. The detection transducer was located at $B$, on the straight tube after the exit of the curve and parallel to the entrance length, whereas the void creation occurred 0.2 m upstream at $A$. For the helical phantom (Fig. 1c), a helix with radius ($R_h$) 0.5 $D_i$ and a pitch ($P_h$) of 7$D_i$ was used with a total of 10 complete revolutions preceded by a straight entrance section of length 0.09 m (not shown in figure). $B$ lay at the straight tube immediately after the exit of the helix to allow placement of the transducer.

Steady flow was generated by an elevated reservoir tank upstream of the phantom. Microbubbles (detailed later) were added to the header tank (0.1 mL into 5 L of gas-saturated water). Bulk flow was measured and controlled by a rotational flowmeter on the outflow arm of the circuit. The phantoms were submerged in a large tank of water to facilitate ultrasound imaging. The straight and curved phantoms were placed in a plane orthogonal to gravity along with the centreline of the helical phantom. The conformation of the curved phantoms was maintained by use of a supporting large-diameter external rigid polyvinyl chloride tube, with apertures cut for the transducers and thermosted to the appropriate geometry. In the helical case, a custom-made steel mandrel was used. All work was performed at a constant room temperature (22°C).

For all experiments, mean velocity ($u_i$) was set at 0.044 m/s. The dynamic viscosity of water $\mu$ and the density of water $\rho$ were respectively taken to be $9.55 \times 10^{-4}$ Pa s and 997.86 kg/m$^3$. The inflow Reynolds number (Re), defined as

$$\text{Re} = \frac{u_i D_i \rho}{\mu}$$

was 275, a physiologically relevant value for a peripheral artery. Furthermore, for the curved vessel, the Dean number (De), defined as

$$\text{De} = \text{Re} \sqrt{\frac{D_i}{2 R_c}}$$

was 37, which is also physiologically relevant.

**Microbubble contrast agent**

Using the formulation outlined by Sheeran et al. (2011), decafluorobutane microbubbles were prepared by the dissolution of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-sn-glycero-
3-phosphatidylethanolamine-polyethyleneglycol-2000 (DPPPE-PEG-2000) and 1,2-dipalmitoyl-3-trimethylammonium propane (chloride salt, 16:0 TAP) in a molar ratio of 65:5:30 and total lipid concentrations of 0.75, 1.5 and 3 mg/mL. The excipient solution comprised propylene glycol, 5% glycerol and 80% normal saline. Microbubbles were then generated via agitation of a 2-mL sealed vial containing 1.5 mL of the resulting solution using a shaker for 60 s.

The microbubble solution generated was sized and counted according to Sennoga et al. (2012) and had a concentration of about $5 \times 10^9$ microbubbles/mL with the average size at 1 μm. In this study, microbubbles were diluted in outgassed water (Mulvana et al. 2012) to a concentration of $2 \times 10^5$ microbubbles/mL.

**Void creation by bubble destruction**

In the present study, microbubble disruption at high ultrasound pressure was harnessed as a technique for injecting a negative indicator by creating a microbubble void within a microbubble-filled vessel. A long burst of 5000 cycles at 2 MHz and a peak-negative pressure of 1.3 MPa was transmitted from the single-element unfocussed transducer to destroy the microbubbles within the intersection volume between the ultrasound field and the vessel lumen. This level of ultrasound transmission is well within the Food and Drug Administration (FDA) safety limit (FDA 2008).

**Ultrasound imaging**

A L12-5 50-mm linear array transducer mounted on a Verasonics Vantage 128 research platform (Verasonics, Redmond, WA, USA) was placed perpendicular to the local centreline of the phantom to image the cross-sectional flow field. The Verasonics system was programmed to send out plane waves of inverted phase, which are capable of specifically detecting microbubbles with high frame rate (Tanter and Fink 2014) and avoiding potential issues with traditional line-by-line scanning (Zhou et al. 2013).

Sixteen plane waves with angles spanning $-18^\circ$ to $18^\circ$ were transmitted at 100 Hz to form an image after coherent compounding. For each pulse sequence, the broadband transducer was driven to transmit repetitively a 5-MHz 1-cycle imaging pulse followed by its phase inverted counterpart, while the radiofrequency echoes were received at a centre frequency of 7.8 MHz and accumulated in the local memory. The radiofrequency data collected were then transferred back to a computer through a high-speed PCI-Express connection, software beam formed into a series of CEUS images and further analysed using MATLAB (The MathWorks, Natick, MA, USA).

**Ultrasound image enhancement**

Before quantification of the mixing, CEUS images were processed as illustrated in Figure 2 to enhance the image quality. The region of interest containing the cross section of the tube was first selected from temporal snapshots and cropped using a circular mask. The regions of interest were then processed using proper orthogonal decomposition to enhance the visibility of the coherent structures that vary in space and time depending on flow geometry (Garg et al. 2013; Holmes et al. 1998). In summary, the images were decomposed into a finite number of proper orthogonal modes by determination of eigenfunctions using the methods of snapshots (Sirovich 1987) and reconstructed with the first $N$ most energetic modes to capture the dominant structure. In this process, incoherent speckle and noise were removed, and the coherent bubble response and microbubble void were emphasised. To further enhance the image, the contrasts of the reconstructed images were enhanced by transforming the grey-scale intensity using a sigmoid function.

**Table 1. Estimated set of parameters for $\tilde{\alpha}(x)$**

<table>
<thead>
<tr>
<th>$a$ (MPa)</th>
<th>$x_a$ (m)</th>
<th>$x_c$ (m)</th>
<th>$b$ (m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3565</td>
<td>0.000576</td>
<td>0.03049</td>
<td>926.121</td>
</tr>
</tbody>
</table>
Computational fluid dynamics

Geometries. Geometries matching the experimental setup illustrated in Figure 1 were used in all the CFD simulations. In all cases, $Re = 275$, and for the curved configuration, $De = 37$ was used, indicating that the flow is fully laminar. A 0.006-m-diameter void (zero bubble concentration) was inserted 0.02 m away from the flow outlet, and the cross-sectional flow patterns were observed at the flow outlet.

Governing equations. Flow. Water was treated as an incompressible Newtonian fluid. Specifically, flow was modelled using the steady incompressible Navier–Stokes equations for a fluid with a constant viscosity, which can be written as

$$\nabla \cdot u = 0$$

(3)

$$(u \cdot \nabla)u = -\frac{1}{\rho} \nabla p + \frac{\mu}{\rho} \nabla^2 u$$

(4)

where $\mu$ is the viscosity of water, $\rho$ is the density of water, $u$ is the 3-D velocity (vector) field and $p$ is the pressure field. The values of $\mu$ and $\rho$ were chosen to match the in vitro experiment, resulting in identical $Re$ and $De$ numbers as above.

Microbubbles. Microbubbles were treated as a continuum species dissolved in water rather than a discrete phase. Specifically, microbubble transport was modelled using the time-dependent advection equation for a passive scalar, which can be written as

$$\frac{dc}{dt} = -u \cdot \nabla c$$

(5)

where $u$ is the 3-D velocity (vector) field, and $c$ is the non-dimensional microbubble concentration. Use of the advection equation to model microbubble transport is justified in this instance given the very large Peclet number.

Boundary condition. Flow. A steady-state constant boundary-normal parabolic flow profile with a spatially averaged velocity $u_i = 0.044$ m/s (equivalent to 1.3 mL/s) was applied at the inlet. A constant (and

Fig. 3. Ultrasound peak-to-peak pressure profile (dashed line) and the microbubble–void transition profile (dot-dash line) reconstructed from the fitted ultrasound beam profile function (solid line). Note that the left y-axis corresponds to the measured peak-to-peak ultrasound pressures and the fit function $\tilde{a}(x)$, whereas the right y-axis corresponds to the microbubble–void transition profile.

Fig. 4. Cross-sectional view of the O-grid used. The section was swept along the centrelines of each vessel to produce hexahedral cells.
microbubbles. For each configuration, a steady-state constant concentration of 1.0 was applied at the inlet, and a no-stress boundary condition was applied at the outlet. A zero-flux boundary condition was applied at the walls, modelling the walls as impermeable to microbubbles.

Initial condition. Flow. For each configuration, the flow solver was initialised with a zero-velocity and zero-pressure field.

Microbubbles. The initial microbubble concentration field had a value of 1.0 everywhere except for the region of microbubble destruction, where a value of 0 was imposed. A smooth transition between the two regions was applied to take into account the ultrasound beam pressure profile. Specifically, the measured beam pressure profile was fitted to a double logistic step function \( \sigma(x) \) using a least-squares method to minimise error between the fit and the measured values. The generic logistic function \( \sigma(x) \) is defined as

\[
\sigma(x) = \frac{1}{1 + e^{-bx}}
\]

whereas the double logistic step function is

\[
\tilde{\sigma}(x) = a[\sigma(x-x_s) - \sigma(x-x_e)]
\]

where \( a, x_s, x_e, \) and \( b \) parameterise \( \tilde{\sigma}(x) \). Table 1 lists the values of these parameters estimated using a least-squares method.

Figure 3 illustrates \( \tilde{\sigma}(x) \) fitted to the measured ultrasound beam profile and the microbubble–void transition, defined as \( 1 - \tilde{\sigma}(x) \), having the same \( x_s, x_e \) and \( \sigma \), and \( a = 1.0 \).

Computational method

Hexahedral meshes were produced for each of the three geometries using Star-CCM + Version 9.02.005 (CD-Adapco, Melville, NY, USA), as illustrated in Figure 4. The grids contained approximately 8.3 M, 12.7

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>Longitudinal View</th>
<th>Cross-sectional view</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>2.3</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>3.0</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Fig. 5. Arrival of the microbubble void observed by high-frame-rate contrast-enhanced ultrasound at different time points within an image plane along the vessel axis (left) and in the cross-sectional view (right). The elliptic dark wavefront is the front of the evolved void (absence of bubbles) travelling along the vessel.

arbitrary) pressure was applied at each outlet. A zero-velocity no-slip condition was applied at the walls, which were assumed to be rigid.

Microbubbles. For each configuration, a steady-state constant concentration of 1.0 was applied at the inlet, and a no-stress boundary condition was applied at the outlet. A zero-flux boundary condition was applied at the walls, modelling the walls as impermeable to microbubbles.

Initial condition. Flow. For each configuration the flow solver was initialised with a zero-velocity and zero-pressure field.

Microbubbles. The initial microbubble concentration field had a value of 1.0 everywhere except for the region of microbubble destruction, where a value of 0 was imposed. A smooth transition between the two regions was applied to take into account the ultrasound beam pressure profile. Specifically, the measured beam pressure profile was fitted to a double logistic step function \( \tilde{\sigma}(x) \) using a least-squares method to minimise error between the fit and the measured values. The generic logistic function \( \sigma(x) \) is defined as

\[
\sigma(x) = \frac{1}{1 + e^{-bx}}
\]

whereas the double logistic step function is

\[
\tilde{\sigma}(x) = a[\sigma(x-x_s) - \sigma(x-x_e)]
\]

where \( a, x_s, x_e \), and \( b \) parameterise \( \tilde{\sigma}(x) \). Table 1 lists the values of these parameters estimated using a least-squares method.

Figure 3 illustrates \( \tilde{\sigma}(x) \) fitted to the measured ultrasound beam profile and the microbubble–void transition, defined as \( 1 - \tilde{\sigma}(x) \), having the same \( x_s, x_e \) and \( \sigma \), and \( a = 1.0 \).

Computational method

Hexahedral meshes were produced for each of the three geometries using Star-CCM + Version 9.02.005 (CD-Adapco, Melville, NY, USA), as illustrated in Figure 4. The grids contained approximately 8.3 M, 12.7
M and 16.1 M cells (M = million) in the straight, curved and helical configurations, respectively. Grid independence of the solutions is discussed in the Appendix 1.

Solutions for the velocity field and the microbubble concentration were obtained using the following procedure:

- Each simulation was initialised with zero velocity and pressure and run until convergence with the segregated steady-state solver.
- Each steady-state flow solution (velocity and pressure field) was then used as the initial condition for the implicit unsteady solver, which advanced each simulation 15 s. During this time, the segregated solver was kept frozen, and only microbubble advective transport was solved for. The time step was 0.01 s, and each step was solved until residual convergence.

The experimental data obtained in this study will be available on request by emailing Ultrasound-Imaging-Group@imperial.ac.uk.

RESULTS

Microbubble void concentration

Figure 5 illustrates the arrival of the microbubble void observed by high-frame-rate CEUS with two different views. In the image plane along the vessel axis, it can be seen that the initial (flat) wavefront at the site of void creation has developed into an elliptic wavefront and travels from left to right, whereas the cross-sectional view depicts the evolution of a circular ring from the centre of the tube toward the tube wall.

Figure 6 illustrates the qualitative comparison of the temporal analysis of microbubble concentration acquired from the CFD solution and ultrasound experiment. It is clear that the structures of the microbubble void acquired from ultrasound images are very similar to the CFD solutions in all cases: a circular ring growing progressively from the centre of the tube was observed in a straight tube, two symmetric vortices that progressively increase in size were detected in a curved structure and two asymmetric vortices were captured in a helical structure. However, it should be noted that the void structures obtained from ultrasound images in the helical case are not as distinctive as those from the CFD solutions. This may be a result of the high degree of mixing in a helical structure and the sensitivity of void detection being beyond ultrasound resolution.

Mixing evaluation

An entropic measure can be used to quantify the degree of mixing. Specifically, according to Cookson et al. (2009), the degree of mixing in the case where there are two species (here microbubble and microbubble void) can be quantified as

$$S = \frac{\sum_{j=1}^{N} c_j \ln(c_j) + (1-c_j) \ln(1-c_j)}{S_{max}}$$

(8)
where $N_b$ is the number of bins into which the domain is divided, $c_j$ is the microbubble concentration in each bin and $S_{\text{max}} = N_b \ln(1/2)$, which corresponds to the case in which $c_j = 1/2$ in every bin.

Time–entropy curves as illustrated in Figure 7 are computed to evaluate the mixing properties. In all cases, results from two independent ultrasound experiments are compared with the CFD solution. As the mixed void region passes through the analysis plane, $S$ increases to a maximum before decaying as the mixed void region leaves the analysis plane. Similar trends are observed in both ultrasound experiments and CFD solutions, but it should be noted that the entropic measure $S$ decreases at a much faster rate in the experimental cases. This disagreement is due to resolution limits on the experimental technique compared with those with the CFD approach.

The time–entropy curves provide useful information to help quantify the degree of mixing. Here we elect to use the peak entropy obtained over each time series ($S_p$) to quantify the degree of mixing induced by each geometry. Figure 8 indicates the value of $S_p$ for each geometry. It can be observed that there is good agreement between the computational and experimental results. It can also be
observed that the level of mixing $S_p$ increases from the straight, to the curved to the helical geometries, in line with expectations.

**DISCUSSION**

A new ultrasound imaging technique for non-invasively visualising and quantifying flow mixing in optically opaque media has been developed using high-frame-rate ultrasound imaging and controlled microbubble destruction. Initial *in vitro* evaluation of the system with three different geometries compared with reference CFD solutions has indicated the potential of this technique.

Significant advantages of the proposed technique include elimination of the need for local injection of an indicator, which can be highly invasive and may disturb the flow and the properties of which may differ from those of the medium into which it is injected. Furthermore, the technique has the potential to allow repeated measurements at various ultrasound-accessible sites after a single injection of indicator and offers a high degree of control of the “indicator injection” in both time and space. Microbubbles can be introduced into the circulation through distant peripheral intravenous injection and then destroyed essentially instantaneously at will with precise spatial and temporal control. The microbubbles are small (1 to $7 \times 10^{-6}$ m) with negligible mass compared with surrounding fluid and occupy a very small percentage of volume (volume fraction typically $\leq 0.01\%$ [Tang and Eckersley 2006; Tang et al. 2011a]) and have very high Peclet numbers. Therefore, they do not significantly disturb the flow or diffuse far enough within the flow to affect the assessment of flow mixing. Compared with other modalities such as MRI, DSA and CT, which often involve invasive procedures, the proposed method offers a non-ionising, rapid, real-time and affordable system that can visualise qualitatively the flow field and quantify mixing both *in vitro* and potentially *in vivo*. The ultrasound output parameters used in this study for the destruction of microbubbles are within the FDA safety limit (FDA 2008).

Although the proposed method has been found to be feasible in an *in vitro* study, it can be further refined. The spatial resolution and signal-to-noise ratio can be further improved by optimisation of ultrasound system parameters such as frequency and acoustic pressure. In this work, the system acquired images at 100 fps which is sufficient for the relatively low flow velocity in this study. To deal with the high flow rate *in vivo* (Leow et al. 2015), the frame rate can be significantly increased because the
imaging system is capable of acquiring at thousands of frames per second (Leow et al. 2015), which will create much larger amount of data than traditional ultrasound scanners. From a clinical application standpoint, real-time processing would be desirable to provide immediate qualitative and quantitative assessment. This is made possible with recent advances in Graphics Processing Unit that can provide parallel processing of ultrasound data and can deliver real-time measurements.

Assessment of mixing is valuable in studying fluid-wall mass transport which may play a major role in the initiation and development of vascular pathology such as atherosclerosis and intimal hyperplasia (Caro 2009; Tarbell 2003). Using a non-invasive ultrasound method and the technique proposed, not only can we qualitatively observe the mixing and flow field, but we can also quantitatively evaluate the degree of mixing using a scalar measure. This is attractive, as real-time clinical application of the technique could allow correlation of mixing with cardiovascular geometry, flow features and clinical outcome. This leads to the possibility of designing vascular interventions, such as bypass grafts, arterial stents and arteriovenous fistulae, to optimise outcomes. Equally, the technique presented here may assist in the early diagnosis and management of vascular disease, incorporating indicators of flow into the assessment of lesions.

Future studies with the technique, beside further optimisation of the ultrasound parameters, include assessment of non-steady flow and with different Reynolds numbers and imaging at different sites relative to bubble destruction site. Given promising results, the study will be extended to preclinical in vivo measurements.

CONCLUSIONS

In this study, visualisation and quantification of flow mixing in optically opaque media using plane wave ultrasound imaging and controlled microbubble destruction were studied. Initial experiments on different flow geometries agree well with independent CFD simulations and indicate the potential of this technique for cardiovascular applications in vivo.

Acknowledgments—Meng-Xing Tang acknowledges the funding from UK EPSRC (EP/M011933/1 & EP/K503733/1). Chee Hau Leow is supported by a postgraduate scholarship from the Public Service Department of Malaysia.—The authors are grateful for support from the National Institute for Health Research Imperial Biomedical Research Centre, the Garfield Weston Foundation, CD-Adapco, and the British Heart Foundation (FS/14/19/30609).

REFERENCES


Food and Drug Administration (FDA). Guidance for industry and FDA staff—Information for manufacturers seeking marketing clearance of diagnostic ultrasound systems and transducers. Silver Spring, MD: Author; 2008.


APPENDIX

GRID CONVERGENCE

Grid independence was achieved for the helical case, which was considered to be the most challenging in terms of resolving the dynamics. Specifically, computations were performed on increasingly finer grids until evolution of the entropic measure of mixing was seen to converge and become grid independent.

Time–entropy curves for the helical geometry with different grid resolutions are provided in Figure 9. Snapshots of microbubble concentration at the imaging plane of the helical geometry for different grid resolutions are provided in Figure 10. Variation of the peak entropic measure of mixing ($S_p$) with grid resolution for the helical geometry is illustrated in Figure 11. It is clear that the solutions become grid independent as resolutions increase. Specifically, $S_p$ varies only by 2% when the grid resolution is increased from 16.1 M to 21.7 M cells. Hence all simulations were undertaken on a grid of 16.1 M cells for the helical case, and an equivalent resolution was adopted in the straight and curved geometries.