Ultrasound in Med. & Biol., Vol. 41, No. 11, pp. 2926–2937, 2015

Printed in the USA
0301-5629
http://dx.doi.org/10.1016/j.ultrasmedbio.2015.06.023

● *Original Contribution*

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

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(*Received* 26 *March* 2015; *revised* 16 *June* 2015; *in final form* 30 *June* 2015)

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

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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 $D = 7.6 \times 10^{-14}$ m^2/s and a Peclet number Pe $= 3.5 \times 10^{9}$ 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