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● *Original Contribution*

QUANTITATIVE GUIDELINES FOR THE PREDICTION OF ULTRASOUND CONTRAST AGENT DESTRUCTION DURING INJECTION

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Abstract—Experiments and theory were undertaken on the destruction of ultrasound contrast agent microbubbles on needle injection, with the aim of predicting agent loss during *in vivo* studies. Agents were expelled through a variety of syringe and needle combinations, subjecting the microbubbles to a range of pressure drops. Imaging of the bubbles identified cases where bubbles were destroyed and the extent of destruction. Fluid-dynamic calculations determined the pressure drop for each syringe and needle combination. It was found that agent destruction occurred at a critical pressure drop that depended only on the type of microbubble. Protein-shelled microbubbles (sonicated bovine serum albumin) were virtually all destroyed above their critical pressure drop of 109 ± 7 kPa Two types of lipid-shelled microbubbles were found to have a pressure drop threshold above which more than 50% of the microbubbles were destroyed. The commercial lipid-shelled agent Definity was found to have a critical pressure drop for destruction of 230 ± 10 kPa; for a previously published lipid-shelled agent, this value was 150 ± 40 kPa. It is recommended that attention to the predictions of a simple formula could preclude unnecessary destruction of microbubble contrast agent during *in vivo* injections. This approach may also preclude undesirable release of drug or gene payloads in targeted microbubble therapies. Example values of appropriate injection rates for various agents and conditions are given. (E-mail: rmanasseh@swin.edu) **© 2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Microbubbles, Ultrasound contrast agent, Pressure drop, Injection, Destruction.**

INTRODUCTION

Ultrasound contrast agents have been in clinical use for more than 20 y (de Jong et al. 2002; Grinstaff and Suslick 1991; Lindner 2004; Qin and Ferrara 2007). In addition to their use as a passive "dye," there is presently great interest in "targeting" microbubbles with antibodies or similar biochemical moieties so that tissues in specific disease states can be identified on a scan (Anderson et al. 2011; Dayton and Ferrara 2002; Doinikov et al. 2009; Martin et al. 2007; Zhao et al. 2006). Intravenously injected microbubbles have been found to improve the condition of acute stroke patients (Cintas et al. 2004; Daffertshofer and Hennerici 2003; Molina et al. 2006; Perren et al. 2008). Microbubbles are also increasingly proposed for drug delivery (Choi et al. 2007; Ferrara et al. 2007; Forbes et al. 2008; Raymond et al. 2008; Rapoport et al. 2009) and gene therapy (*e.g.*, Browning et al. 2011; Delalande et al. 2010; Tsutsui et al. 2004). The bubbles are typically a few microns in diameter and have a polymer, lipid or proteinaceous shell (Borrelli et al. 2012; Stride and Edirisinghe 2008). The shell is very important because without it, Laplace pressure would cause bubble dissolution in a few seconds (Brennen 1995), and as agents are usually intravenously administered, they must survive the pulmonary circulation before being of use for arterial diagnostics or therapeutics.

Failure of the shell therefore limits the lifetime of the agent. The failure of the shell in an ultrasound pulse is

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a dynamic process (*e.g.,* Yasui et al. 2009). However, simple intravenous administration of the agent, particularly in small-animal studies demanding fine needles, often results in steady pressure gradients that can cause failure of the shell (Browning et al. 2011; Talu et al. 2008). Shell rupture would also be relevant to drug delivery (*e.g.,* Browning et al. 2011; Rapoport et al. 2009), when it would be desirable to ensure the shell is ruptured by the ultrasound beam in the target organ and not at the point of injection. In these circumstances, agents other than those commercially available may be required (Borrelli et al. 2012).

Detailed theoretical and experimental studies have been made of the mechanisms of contrast agent destruction (Chomas et al. 2001; King et al. 2010; Yasui et al. 2009). These studies necessarily focus on the destruction of the agent in the oscillating pressure field of the ultrasound beam. However, during the needle injection process, the microbubbles are compressed by high pressure in the syringe barrel, then subjected to an abrupt release of this pressure during their passage through the needle. Depending on the nature of the encapsulating shell material (protein, polymer or lipid), the shell may buckle during the compression stage, then suddenly relax to equilibrium during the pressure release stage. For some agents, the rebound in diameter during the pressure release may cause an over-expansion in diameter, critically thinning the shell; for others, buckling during compression might cause failure. In any case, the magnitude of the pressure drop experienced would seem relevant.

From standard engineering fluid dynamics textbooks (*e.g.,* Streeter and Wylie 1979), the pressure variation experienced by a bubble passing from a syringe barrel into the vasculature has a number of components: the pressure drop resulting from the change in cross-sectional area from the syringe barrel to the needle; the entry loss at the abrupt change in diameter; and frictional losses during flow through the needle and any tubing attached to it. Owing to the presence of energy losses where diameters change abruptly, it is appropriate to use the energy conservation equation, not Bernoulli's equation, which is derived from the principle of momentum conservation (Newton's second law).

Although it is intuitive that both the injection rate and needle gauge should affect bubble destruction, the level of detail reported in the literature varies. For example, Stapleton et al. (2009) and Browning et al. (2011) fully describe the injection conditions, mentioning needle gauges, bolus volume and time over which injection occurred, whereas Willmann et al. (2008) mention bolus volume and injection time and Choi et al. (2007) mention bolus volume.

Talu et al. (2008) found that the extent of destruction of a lipid-shelled microbubble was, as expected, associated with both needle gauge and injection speed, with the percentage of lipid-shelled microbubbles destroyed increasing for finer needles and for higher flow rates. A reduction in either syringe or needle inner diameter was found by Barrack and Stride (2009) to produce large reductions in microbubble concentrations; Barrack and Stride (2009) also found that changes in static pressure were unlikely to be the main mechanism of destruction. Talu et al. (2008) had also found that conditions leading to greater destruction also shifted the bubble population to smaller sizes, a finding corroborated by the results of Browning et al. (2011).

Barrack and Stride (2009) were the first to apply fluid-dynamic calculations to the needle injection problem. They calculated the shear stress resulting from fluid flow; and they also calculated the dynamic pressure caused by fluid flow using Bernoulli's equation, which, as noted above, may not be correct for needle injection. Their comparison of dynamic pressure with static pressure, which they tested in a separate experiment, may have been inappropriate. The key difference during injection is that the dynamic pressure can vary very rapidly. In summary, previous research has not interpreted data in terms of the dynamic pressure drop.

Albunex (Mallinckrodt, Hazelwood, MO, USA) was a first-generation ultrasound contrast agent, now superceded, with a proteinaceous shell made of sonicated human serum albumin and an air core. The destruction pressure of Albunex was estimated by Christiansen et al. (1994) to be between 67 and 530 kPa, as these were two of the three pressures tested. In their experiment, the pressure was applied statically, not *via* an injection process.

The aims of the work described here were to calculate the pressure drop experienced during microbubble injection, based on the elementary fluid dynamics of the syringe injection process, and to experimentally compare the pressure drops causing destruction of protein- and lipid-shelled microbubbles. The hypothesis to be tested is that calculation of the fluid-dynamical pressure drop during injection can predict agent destruction for any desired syringe, catheter and needle combination.

METHODS

Materials and experimental procedure

Microbubbles with a proteinaceous shell of bovine serum albumin (BSA) were prepared by batch sonication (*e.g.,* Gedanken 2008; Grinstaff and Suslick 1991). In general, the present process was the simplest possible, similar to that used in the production of Albunex and