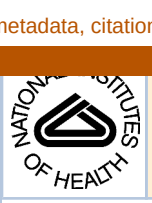

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Needle Size and Injection Rate Impact Microbubble Contrast Agent Population

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Abstract

The most common type of ultrasound contrast agents are encapsulated microbubbles, typically 1–5 microns in diameter. These microbubbles are injected into the bloodstream to provide image enhancement during an ultrasound exam. Due to their compressibility, these microbubbles are inherently sensitive to changes in pressure. For imaging, this is beneficial in that these microbubbles oscillate in an acoustic field and allow imaging systems to detect their response uniquely from tissue. However, this sensitivity also means that microbubbles can be readily destroyed by significant hydrostatic pressure. Injection of these microbubbles through a small-gauge catheter, such as sometimes performed in small animal imaging studies, can result in microbubble destruction. In this manuscript, the effects of microbubble injection through catheters of varying diameter are examined. Our results indicate that the concentration and size distribution of microbubbles can be substantially altered in cases of rapid injection through small needles.

Keywords

microbubble; contrast agent; size distribution; ultrasound; molecular imaging

INTRODUCTION

Ultrasound contrast agents (UCAs) are currently being used in biomedical applications such as radiology and cardiology (Goldberg et al. 2001). These contrast agents are micron-sized gas bubbles which are injected intravascularly during an imaging exam. In response to an acoustic pulse, microbubble contrast agents resonate at a diagnostic ultrasound frequency and improve the contrast between blood and tissue (Villanueva et al. 1995); (Lang et al. 2006), or highlight disease or tissue injury (Lindner 2004); (Dayton et al. 2002). A thin shell of lipid, protein, or polymer is placed around the gas core to stabilize these microbubbles, as unencapsulated gas would diffuse in milliseconds (Dayton et al. 1999). Although first-generation agents utilized an air or nitrogen core, current microbubble agents are typically filled with a high-molecular weight gas such as a perfluorocarbon due to lower gas solubility.

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One of the characteristics of a microbubble contrast agent which makes it highly echogenic is the ability to resonate in an acoustic pressure field. This property is directly related to the shell rigidity (Postema et al. 2007). Commercially available agents produce a strong scattered echo either by oscillating with an intact flexible shell (Chomas et al. 2002); (de Jong et al. 2002), or by bursting, releasing a free gas bubble which oscillates before it dissolves (Bouakaz et al. 2005); (Chomas et al. 2001); (Frinking et al. 2001). Since these agents are sensitive to an acoustic pressure field, they are also sensitive to a hydrostatic pressure field. A large change in hydrostatic pressure will burst many of the microbubbles.

Contrast agent microbubbles are being increasingly used in small animal imaging, particularly with the development of new high-resolution pre-clinical imaging systems (Foster et al. 2002) and the advancement of molecular imaging (Lindner 2004). Although some small researchers utilize jugular vein cannulation for contrast administration (Ellegala et al. 2003), this technique is more complicated and difficult for serial studies. For contrast injection into small animals, or occasionally in in-vitro studies, many researchers utilize small gauge catheters/needles with tail vein injection which can result in a large hydrostatic pressure differential during injection. The literature reports examples of 24 gauge needles used for injection into rat tail veins (Miller et al. 2007), 27 gauge needles utilized for tail vein injection into mice (Howard et al. 2006), and 30 gauge needles used for contrast studies of the lymphatic system (Wisner et al. 2002). Often, researchers do not report infusion rate, although some reported rates include 0.5 mL/kg/min into rats (Miller et al. 2007), as well as “bolus” injection into mice and rats (Choi et al. 2007) (Stieger et al. 2006).

In this study, we examine the sensitivity of lipid-shelled microbubbles to injection through various gauge needles to determine thresholds for contrast injection with minimal bubble disruption. We examine flow rates from 0.6 ml/minute to 0.5 ml/second to simulate ranges from fast infusion to rapid bolus injection. In addition to catheter size the injection rates, we consider microbubble concentration, as it is known that microbubble contrast agents are more stable in a high concentration. Dilutions of lipid shelled microbubble contrast agents utilized for small animal imaging reported in the literature cover a wide range, including ~1:10 to ~1:50 (Miller et al. 2007, Stieger et al. 2006).

MATERIALS AND METHODS

Lipid-encapsulated microbubble contrast agents were prepared as previously described (Borden et al. 2002). Briefly, 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1, 2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (DSPE-PEG2000) (Avanti Polar Lipids; Alabaster, AL) were combined at a molar ratio of 9:1, and suspended in a 0.1 M Tris buffer (Sigma-Aldrich Corp.; Louis, MO). The solution was placed into sonic bath for 45 min and then aliquoted into gas tight vials to which decafluorobutane gas was added (SynQuest Labs; Alachua, FL). Microbubbles were formed by shaking method using a CAPMIX machine (ESPE; Seefeld, Germany) for 45 s.

Microbubble concentrations of approximately $1 \cdot 10^9$, $5 \cdot 10^8$ and $1 \cdot 10^8$ microbubble/ml (equivalent to ~1:10 to ~1:100 for some lipid-encapsulated perfluorocarbon agents (http://www.definityimaging.com/pdf/prescribinginfo.pdf) were used. 23, 27 and 30 gauge needles were used with an addition of 6” polyethylene tubing with diameters matching the needle gauge. 5 different flow rates (0.01, 0.03, 0.1, 0.3, and 0.5 ml/sec) were provided by a high precision Harvard Apparatus (Holliston, MA) syringe pump. The microbubble concentration and size distribution before and after the injection of the microbubbles were measured with an Accusizer (Particle Sizing Systems Inc.; Santa Barbara, CA). All experiments were repeated three times.

RESULTS

The size distribution of microbubbles before injection was approximately 0.95 μm ± 0.75 μm for all concentrations. Results are separated according to injection needle size.

23-gauge needle

Injection through the 23-gauge needle did not affect either the microbubble concentration or the size distribution except for the highest flowrate tested. At a flowrate of 0.5 ml/sec, the percentage of microbubbles destroyed during injection was 70 ± 2 % (Figure 1A) and the mean diameter of the size distribution was reduced by 10 ± 1 % (Figure 2A) for all concentrations.

27-gauge needle

When 27-gauge needle was used at a $1 \cdot 10^8$ microbubble/ml concentration, more than 85 ± 2 % of the microbubbles were destroyed for flowrates higher than 0.1 ml/sec. At a microbubble concentration of $5 \cdot 10^8$ microbubble/ml, only 30 ± 3 % microbubble loss was observed for 0.1 ml/sec; however, further increase in the flowrate above 0.3 ml/sec resulted in more than 95 % microbubble loss. At a microbubble concentration of $1 \cdot 10^8$ microbubble/ml, no destruction was observed up to 0.1 ml/sec, but 82 ± 3 % and 98 ± 2 % microbubble destruction was observed for 0.3 and 0.5 ml/sec, respectively (Figure 1B).

The mean diameter of the size distribution dropped more than 10 % with the use of 27-gauge needle at all microbubble concentrations when the flowrate was more than 0.3 ml/sec. At a flowrate of 0.1 ml/sec, decrease in mean diameter was observed to be 2, 6 and 10 % at $1 \cdot 10^9$, $5 \cdot 10^8$ and $1 \cdot 10^8$ microbubble/ml, respectively (Figure 2B).

30-gauge needle

The 30-gauge needle was observed to be destructive to microbubbles at all flow rates tested at $1 \cdot 10_8$ microbubble/ml concentration. More than 55 % of the microbubbles were destroyed at 0.01 ml/sec, and 96 % of the microbubbles were lost for flowrates 0.1 ml/sec and above. With the use of the 30-gauge needle at both $1 \cdot 10^9$ and $5 \cdot 10^8$ microbubble/ml concentration, 99 % of the microbubbles were destroyed at flowrates of more 0.1 ml/sec (Figure 1C).

Approximately 15 % reduction in size distribution was observed at all microbubble concentrations at flowrates above 0.1 ml/sec (Figure 2C). However, at these flow rates – there were very few contrast agents remaining after being pumped through the catheter.

DISCUSSION

Our results indicate that injection of contrast through a 23-gauge needle was relatively non-destructive to microbubble contrast agents for all concentrations tested at flow rates up to 300 microliters/sec, which would be a very rapid bolus injection. In contrast, we observed that injection through a 30-gauge needle was very destructive (>50% loss in concentration) for contrast agents of $1 \cdot 10^8$ bubbles/ml concentration at all flow rates tested, and very destructive for $5 \cdot 10^8$ and $1 \cdot 10^9$ bubbles/ml at bolus injection rates > 30 microliters per second.

In addition to destruction of microbubbles, a shift in the mean diameter of the population towards a smaller diameter was also observed for cases where microbubble loss during injection was observed. This likely indicated either that large bubbles were destroyed preferentially, or the population diameter was reduced, possibly by forced compression and resulting gas loss. Further investigations will elucidate specific mechanisms for these changes.

This study was largely limited by the fact that only one type of lipid-shelled contrast agent was tested. However, we anticipate similar trends for the stability of other microbubble type contrast agents in response to injection parameters.

Maintaining consistency in microbubble population is important in contrast enhanced ultrasound, particularly in applications where contrast is used for quantitative measurement. This is complicated further by the fact that the contrast intensity is not always a linear function of the microbubble concentration (Porter et al. 2003). One application where experimental consistency might be particularly sensitive to administered concentration is ultrasonic molecular imaging, where intensity of contrast agents retained at a target site provides information as to the expression of a specific molecular marker.

Additionally, microbubble concentration has also been found to play an important role in therapeutic applications, where microbubble concentration has been shown to be related to efficiency of gene transfection (Zhao et al. 2007) and localized heating (Razansky et al. 2006).

Microbubble concentration is also related to the likelihood of bioeffects, where higher concentrations are related to increased cavitation (Tu et al. 2006), and differences in the amount of cavitation based effects may be statistically significant at concentration differences as low as 1% (Sassaroli and Hynynen 2007). Part of this mechanism may be due to the formation of larger bubbles as single smaller microbubbles aggregate and then fuse due to secondary radiation force, which is dependent on concentration (Caskey et al. 2007).

Additionally, microbubble size distribution effects contrast agent behavior. Almost all properties of a microbubble contrast agent are related to diameter, including echogenicity and resonant frequency (de Jong et al. 2002, Morgan et al. 2000), susceptibility to radiation force (Dayton et al. 2002), and potential biodistribution (Talu et al 2008). Thus, size distribution should be maintained in order to preserve experimental or diagnostic consistency.

CONCLUSIONS

Lipid-coated and perfluorocarbon-filled microbubbles are known to be stable ultrasound contrast agents. We have shown that the stability of these microbubbles during a contrast injection depends on needle size, contrast agent concentration, and injection rate. We have illustrated several combinations of parameters which are non-destructive for contrast injection, and parameters which will destroy a large majority of the injected dose. Our results indicate that researchers using contrast injection through a needle or catheter should pay specific attention to the injection conditions in an imaging study to avoid deleterious affects on the microbubble contrast agents and to maintain experimental consistency.

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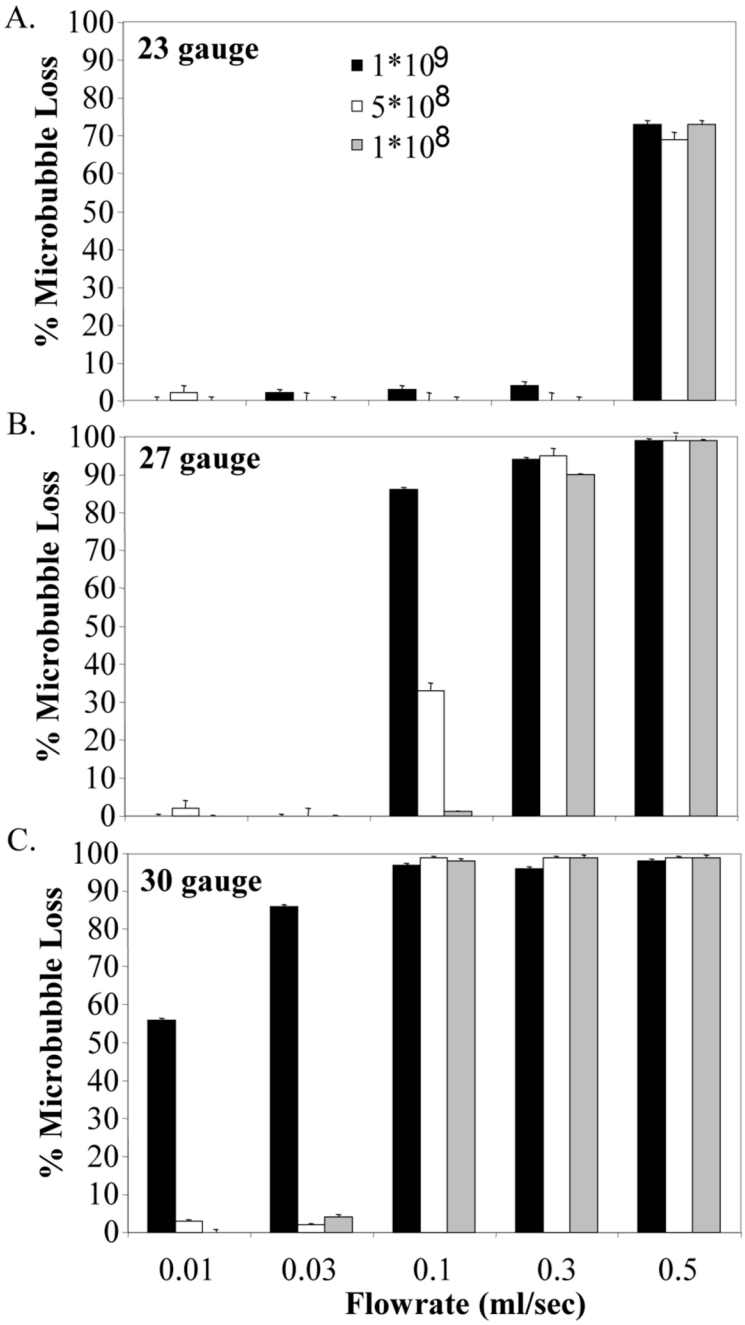

Figure 1.
% Microbubble destruction during infusion through a catheter at as a function of flow rate, concentration, and needle gauge A) 23-gauge, B) 27-gauge, C) 30-gauge.

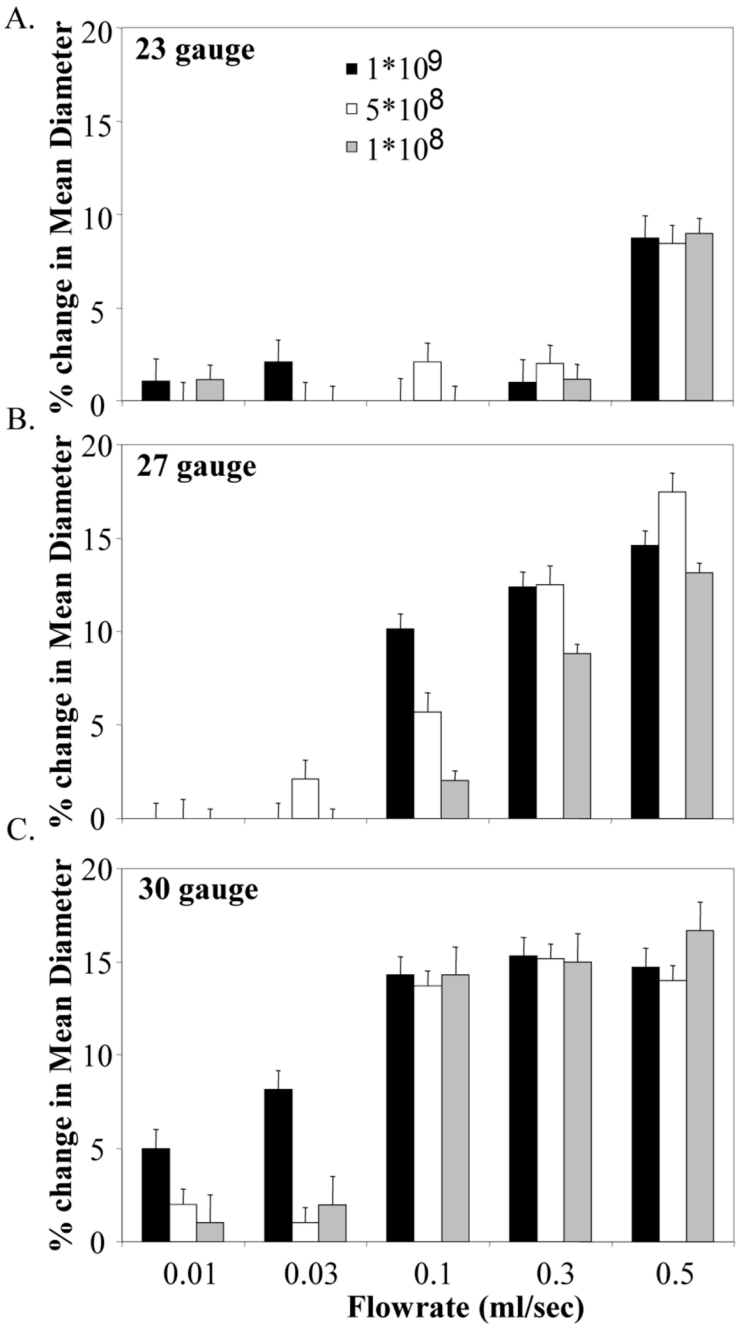

Figure 2.
% change in mean diameter of microbubble population due to injection as a function of flow rate, concentration, and needle gauge A) 23-gauge, B) 27-gauge, C) 30-gauge.