Production rate and diameter analysis of spherical monodisperse microbubbles from two-dimensional, expanding-nozzle flow-focusing microfluidic devices

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Flow-focusing microfluidic devices (FFMDs) can produce microbubbles (MBs) with precisely controlled diameters and a narrow size distribution. In this paper, poly-dimethyl-siloxane based, rectangular-nozzle, two-dimensional (2-D) planar, expanding-nozzle FFMDs were characterized using a high speed camera to determine the production rate and diameter of Tween 20 (2% v/v) stabilized MBs. The effect of gas pressure and liquid flow rate on MB production rate and diameter was analyzed in order to develop a relationship between FFMD input parameters and MB production. MB generation was observed to transition through five regimes at a constant gas pressure and increasing liquid flow rate. Each MB generation event (i.e., break-off to break-off) was further separated into two characteristic phases: bubbling and waiting. The duration of the bubbling phase was linearly related to the liquid flow rate, while the duration of the waiting phase was related to both liquid flow rate and gas pressure. The MB production rate was found to be inversely proportional to the sum of the bubbling and waiting times, while the diameter was found to be proportional to the product of the gas pressure and bubbling time. © 2013 American Institute of Physics.

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I. INTRODUCTION

Controlled production of bubbles has a wide array of applications including: material synthesis,¹ foam production,² micro-mixing,³ sorting,⁴ and medical imaging.⁵ A large area of study is the production of microbubbles (MBs)—low solubility micrometer-sized gas bubbles stabilized with a shell material (e.g., lipid, protein, or polymer) that acts as a surfactant and diffusion barrier.⁶ MBs are the most common ultrasound contrast agent and can be used to enhance anatomic, functional and molecular imaging, as well as drug delivery.⁵ MBs are designed to be less than 10 μm in diameter in order to optimize the acoustic response within clinically relevant ultrasound frequencies (i.e., 1–20 MHz)⁷ and decrease the risk of vascular emboli.^{8,9} Agitation, either mechanical shaking¹⁰ or sonication,^{6,11,12} is currently the most common method for producing MBs. Agitation methods can produce large quantities of MBs (e.g., $\approx 10^{10}$ MB/batch) rapidly.¹³ The diameter distribution of the resulting MB population is highly polydisperse (i.e., polydispersity index or standard deviation/mean, up to 150%),¹³ however, resulting in reduced image contrast compared to monodisperse populations of MBs.^{7,14,15} Various size separating techniques^{16–18} have been developed to sort polydisperse MBs into sub-populations with narrower size distributions, but this adds complexity and reduces overall yield. In contrast, microfluidic devices can produce monodisperse MBs with a polydisperse index as low as 2%.¹⁹

Unlike agitation methods, microfluidic devices produce microbubbles one at a time in a serial process, allowing production rates and diameters to be measured directly using high speed video microscopy.^{19,20} Alternatively, a Coulter counter can be used to determine MB size

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distribution and concentration after production. However, neither of these methods is suitable for MB characterization in real-time applications—such as *in situ* produced MB applications²¹—since MB production rate and diameter would need to be measured every time there is a change in input parameters. For ultrasound contrast, real-time knowledge of the MB diameter can help guide selection of the imaging frequency—MB resonance frequency is related to MB diameter—as well as ensure safety by minimizing the production of MBs with diameters that may pose an emboli risk. For drug delivery, estimates of MB production rate can be used to calculate encapsulated drug dose. Consequently, reliable models that can predict MB diameter and production rates in response to changes in geometry, gas pressure, and liquid flow rates are important for facilitating widespread adoption of microfluidic devices as they can guide flow-focusing microfluidic devices (FFMD) development and operation.

Several classes of microfluidic devices have been investigated for the production of monodisperse MBs, including: co-flow (coaxial capillaries),^{22–24} cross-flow (T-junction),^{25,26} coaxial electrohydrodynamic atomization (CEHDA),^{26,27} “ink jet printing,”²⁸ and flow-focusing.^{19,29–37} FFMDs, including capillary flow-focusing^{29,31} and expanding-nozzle flow-focusing,¹⁹ have the advantage of producing smaller monodisperse MBs ($d_b \leq 5 \mu\text{m}$) as compared with other techniques.³⁸ Two-dimensional (2-D) FFMDs with uniform height and planar rectangular cross-section channels have an additional practical advantage in that they can be cast from polydimethyl-siloxane (PDMS) using microphotolithographically processed epoxy molds.³⁹ Thus, FFMDs can be produced using an inexpensive, simple, and robust fabrication process. Consequently, in this paper, we seek to develop a relationship between the input parameters and the production rate, and diameter of MBs produced via a 2-D expanding-nozzle FFMD design.

In the expanding-nozzle FFMD design, an expanding nozzle links the gas and liquid junction with the outlet¹⁹ (Fig. 1). This geometry results in the development of a large shear stress at the tip of the gas cone⁴⁰ and MB pinch-off. At certain gas and liquid flow rate combinations, this gas pinch-off condition results in the production of highly monodisperse populations of MBs.

Previous studies^{23,29–31,41} have shown that the breakup frequency of a gas jet into MBs in a capillary flow-focusing device with a circular cross-section nozzle and a constant gas flow rate can be described by

FIG. 1. Expanding-nozzle FFMD with $5.4 \mu\text{m}$ -wide, $20.3 \mu\text{m}$ -high nozzle (measured results). (A) Mask design of the custom FFMD, which includes one gas and two liquid inlets with support posts to prevent channel collapse. (B) Enlarged view of gas and liquid junction showing filters on the inlets. (C) Enlarged view of the filter and reservoir for trapping particulates. Filter channels are scaled with nozzle size, w . (D) Enlarged view of the expanding nozzle. Flare angles for the liquid channel and expanding nozzle are θ_l and θ_b , respectively. The channel widths for the liquid and gas channels are w_l and w_g , respectively.

$$\omega \propto \frac{Q_l}{d_j D^2}, \quad (1)$$

where ω is the breakup frequency, Q_l is the liquid flow rate, d_j is the diameter of the gas jet at the nozzle, and D is the nozzle diameter. This relationship was extended to co-flow devices with a square cross-section operating at high Reynolds number ($Re = \rho ul/\mu$, where ρ , μ , u , and l are the fluid density, dynamic viscosity, mean velocity of flow, and characteristic length, respectively) and Weber number ($We = \rho u^2 l/\sigma$, where σ is the surface tension coefficient) and low gas to liquid flow rate ratios ($Re \geq 100$, $We \gg 1$, and $Q_g/Q_l < 0.03$, where Q_g is gas flow rate).²⁰ For FFMDs with a short-channel nozzle and a rectangular cross-section generating Tween stabilized MBs at low Reynolds and capillary number flow conditions ($Ca = \mu u/\sigma$) ($Re \in [0.01, 10]$, $Ca \in [0.001, 0.1]$), Garstecki *et al.*^{32,35} showed that the time that the gas thread stayed open at the nozzle (i.e., τ) was inversely proportional to liquid flow rate

$$\tau \propto \frac{1}{Q_l}. \quad (2)$$

Regarding size, Gañán-Calvo *et al.*^{29,31} developed a universal scaling law to predict MB diameter from a capillary flow-focusing device with a circular cross-sectional nozzle operating at high Reynolds and Weber numbers flow conditions ($Re \in [50, 1400]$ and $We \in [40, 1000]$)

$$\frac{d_b}{D} \propto \left(\frac{Q_g}{Q_l} \right)^{\frac{2}{5}}, \quad (3)$$

where d_b is the MB diameter and $2/5$ is an empirically determined exponent. Recently, Castro-Hernández *et al.*²⁰ derived a scaling law for 2-D co-flow devices with a square cross-section nozzle that was similar to Eq. (3) except the exponent was $5/12$ (flow conditions were: $Re \geq 100$, $We \gg 1$, and $Q_g/Q_l < 0.03$). Garstecki *et al.*^{32,35} reported a different scaling law for the volume of a MB produced in a short-channel nozzle FFMD with rectangular cross-section

$$V_b \propto \frac{P}{\mu Q_l}, \quad (4)$$

where V_b is the volume of MB and P is gas pressure. Garstecki *et al.*³⁴ also observed that the transition of MB production from monodisperse to polydisperse was related to the transition of flow conditions from low Reynolds and capillary numbers ($Re \in [0.01, 10]$ and $Ca \in [0.001, 0.1]$) to high Reynolds and Weber numbers ($Re \in [50, 1400]$ and $We \in [40, 1000]$). However, to the best of the authors' knowledge, no model has been developed to describe MB production rate and diameter for 2-D expanding-nozzle FFMDs.

In this paper, 2-D expanding-nozzle FFMDs were fabricated and MB diameter and production rates were measured over a range of gas pressures and liquid flow rates. In order to facilitate comparison with previous work, Tween—a surfactant and widely used shell material^{20,32–34}—was used to stabilize the MBs. A relationship between MB production rate and diameter as a function of input parameters was subsequently developed.

II. MATERIAL AND METHODS

A. Microfluidic device fabrication

Soft lithography techniques were used to fabricate the 2-D expanding-nozzle FFMDs.^{40,42} Negative photoresist (SU8-3025, Microchem, Newton, MA) was spin-coated onto a silicon wafer to a height of $20 \mu\text{m}$. Standard photolithography was used to pattern the wafer with a custom $0.1 \mu\text{m}$ spot-size quartz mask (Microtronics Inc, Newtown PA). In this design, the expanding nozzle had a 50° flare angle (Fig. 1(d), θ_b), the liquid channels intersected the gas channel at a 90° angle, and the two liquid channels had a 25° flare angle (Fig. 1(d), θ_l) at the gas and

liquid junction.^{43–45} Channel widths for the liquid and gas phase were 50 and 35 μm , respectively^{19,45} (Fig. 1(d), w_l and w_g). Filters were placed immediately after all three inlets to prevent particulates from clogging the nozzle (Fig. 1(b)). 160 μm diameter support posts were placed throughout the channels to prevent collapse in the thickness direction⁴⁶ (Fig. 1(a)). PDMS (Sylgard 184, Dow Corning, Midland, MI), with a 10:1 prepolymer to curing agent ratio, was cast onto the custom designed SU-8 mold and then cured at 80 °C for 30 min. The PDMS layer was then plasma treated (March II, Nordson March, Concord, CA) for 30 s at 25 W and bonded to glass slides. Immediately after bonding, the device was immersed in deionized (DI) water to maintain hydrophilicity of the channels.⁴² Finally, polytetrafluoroethylene (PTFE) tubing (Cole Parmer, Vernon Hills, IL) was inserted into the gas and liquid inlets and sealed using epoxy (Hysol RE2039 and HD3561, Henkel Corp. Mooresville, NC). To maintain a constant pressure drop across all three inlets while providing smooth and pulseless gas and liquid flow, all tubing was 15 cm long with a 0.76 mm outer diameter.

B. Microbubble production

A gas phase consisting of pre-purified nitrogen gas (99.998%) (GTS Welco Richmond, VA) and a liquid phase consisting of 2% (v/v) Tween 20 surfactant (Sigma-Aldrich St. Louis MO)^{32–34} in DI water were used to produce MBs. The input pressure of the gas phase ranged from 6.9–103.4 kPa and was set and monitored using a two-stage regulator (VTS 450D Victor Equipment Company Thermodyne, St. Louis, MO) and a digital manometer (06-664-21 Fisher Scientific Pittsburgh, PA). The liquid phase was supplied via a syringe pump (PHD 2000, Harvard Apparatus, Holliston, MA) at flow rates ranging from 5–120 $\mu\text{l}/\text{min}$. To characterize MB production, the gas pressure was held constant while the liquid flow rate was stepped in 0.5 $\mu\text{l}/\text{min}$ increments until the FFMD no longer produced MBs. The expanding-nozzle FFMDs were allowed to equilibrate for 5 min after any change in gas pressure or liquid flow rate. MB diameter and production rate were measured using an inverted microscope (IX71, Olympus, Center Valley, PA) connected to a high speed framing camera (SIMD24, Specialised Imaging, UK) operating at up to 20 million frames per second. The device was washed and stored in DI water after each experiment to maintain the hydrophilicity of the PDMS channel surface. Repeatability of experiments was tested by repeating diameter and production measurements over several days using the same input parameters.

C. Filter characterization

To determine the smallest particulate that could be trapped by the filter, uniform microspheres (Polysciences, Inc., Warrington, PA) of increasing size were flowed through the inlets. The effect of filter blockage on MB production was characterized by blocking an increasing percentage of filter channels with the microspheres prior to MB production. MB diameter and production rate were then measured and compared at several gas pressure and liquid flow rate combinations.

D. Finite element simulations

Finite element simulations (COMSOL MULTIPHYSICS, COMSOL Inc., Burlington, MA) were performed to evaluate the effects of the filters and posts on gas and liquid flow at their respective inlets within the FFMD. A full 3-D model was constructed using dimensions measured from the final fabricated FFMD. To simplify the simulation, however, the flows were modeled separately. Flow was modeled with the single phase laminar flow physics module. Laminar flow was selected as the calculated Reynolds numbers were below the threshold for turbulence ($\text{Re} < 2000$).⁴⁷ The gas/liquid interface in each simulation was modeled as a wall with a slip-boundary condition—only velocity normal to the boundary was set to zero. A no-slip boundary condition was applied to all other walls.⁴⁸ The geometry was meshed using free tetrahedral elements with a minimum element size of 1.02 μm . A parallel sparse direct linear solver, MUMPS,⁴⁹ was used to calculate the final results.

III. RESULTS AND DISCUSSION

A. Device fabrication and simulation

Scanning electron microscope images of SU-8 molds indicated that the photoresist was fully developed, and all the structures had vertical sidewalls. The measured nozzle width (w) of the final PDMS cast FFMDs was $5.4\ \mu\text{m}$ when using $1.0\ \mu\text{m}$ -wide nozzle molds. The width of liquid and gas channels (w_l and w_g , respectively) were $2.1\ \mu\text{m}$ wider than the mask. Channel height (h) was $20.3\ \mu\text{m}$.

The finite element model predicted a maximum gas pressure drop of $0.13\ \text{Pa}$ along the tubing and $398.6\ \text{Pa}$ from the gas inlet to the nozzle when a maximum gas pressure of $45\ \text{kPa}$ was applied, indicating that the inlet filters and channel posts do not introduce a significant increase in resistance. The gas pressure drop increased to $511.3\ \text{Pa}$ and $743.7\ \text{Pa}$ when 50% and 90% of the filter channels were blocked *in silico*, respectively. The difference between the pressure just before the nozzle, as simulated in the model, and pressure measured by the manometer was less than 1%, suggesting that the manometer provided an accurate measure of the pressure being applied to the nozzle. No difference in liquid flow rate was observed at the nozzle as a result of the filters or support posts. The magnitude of the maximum shear stress was observed to decrease 18% as the flare angle of the expanding nozzle was increased from 20° to 160° at a constant nozzle width. When the flare angle of the expanding nozzle was smaller than 20° , the location of the maximum shear stress moved downstream of the nozzle orifice. Doubling the nozzle width decreased the magnitude of the maximum shear stress by 28%.

B. Flow conditions and experimental results

Liquid properties, gas pressure, liquid flow rate and Reynolds, Weber, and capillary numbers over which the FFMD was stably operated are listed in Table I.

When calculating Reynolds and Weber numbers, the characteristic length was set as the hydraulic diameter (D_h) of the rectangular cross section at the nozzle⁴⁷

$$D_h = \frac{2hw}{h+w}, \quad (5)$$

where h and w are the height and width, respectively, of the channel at the nozzle.

Microspheres less than $3\ \mu\text{m}$ in diameter passed freely through the filters while particles greater than $10\ \mu\text{m}$ were completely trapped by the filter. Diameters in between were partially trapped by the filter (e.g., 50% of $6\ \mu\text{m}$ beads were trapped). The production rate and diameter of MBs produced using an FFMD with blocked filters changed by less than 13% and 3%, respectively, when up to 90% of the channel filters were blocked with $10\ \mu\text{m}$ diameter beads.

The highest gas pressure the FFMD could withstand was $103\ \text{kPa}$. This pressure and a liquid flow rate of $117\ \mu\text{l}/\text{min}$ produced the smallest monodisperse MBs at the highest production

TABLE I. Flow conditions and experimental results.

Property	Value
Liquid viscosity (mPa s) ^{50,51}	0.9
Surface tension (mN/m) ^{35,51}	39.5
Gas pressure range (kPa)	20.7–45.5
Liquid flow rate ($\mu\text{l}/\text{min}$)	32.0–64.0
Reynolds number (Re)	50.0–100
Weber number (We)	5.0–30
Capillary number (Ca)	0.1–0.25
MB diameter (μm)	20.5–4.5
MB production rate (MB/s)	$(0.022\text{--}4) \times 10^5$

rate— $2.9\text{ }\mu\text{m}$ diameter with a polydispersity index of 3.4% at 2×10^6 MBs per second (MB/s). The FFMD was unstable under these parameters, however, and production could not be maintained for more than one minute. While the exact mechanism for the instability has not been elucidated, we hypothesize that random fluctuations in the input parameters and PDMS compliance caused the FFMD, which was already operating near its limit, to cease production (Regime 5 in Fig. 2). At high liquid flow rates, these small random increases might be enough to overcome the gas pressure and stop MB production. Once the FFMD stopped producing MBs, decreases in flow rate larger than the random fluctuations were then needed to restore production, making production unstable. In addition, the pressures required to maintain high flow rates could cause the compliant PDMS to distort. This distortion might lead to a build-up in liquid pressure, which again could overcome gas pressure leading to the cessation of MB production. However, by massively parallelizing MB production^{15,21,52–55}—either through FFMDs with multiple nozzles or multiple FFMDs operating simultaneously—sufficient quantities of microbubbles could be produced in a reasonable time frame (100 FFMDs producing 10^5 MB/s could produce 10^9 MBs in 100 s).

Finally, the effect of the choice of gas on MB production—either nitrogen, PFB, or oxygen—was investigated. The diameter and production rate of MBs produced using each gas was measured across gas pressures ranging between 41.4 and 55.2 kPa and liquid flow rates ranging between 50 and 80 $\mu\text{L}/\text{min}$. The choice of gas did not significantly affect MB production dynamics, with $<6\%$ and $<10\%$ change in diameter and production rate observed across all input parameters. Previous reports,^{56,57} however, suggest that the choice of gas may affect the stability and monodispersity of the MBs immediately after production and during storage.

C. Microbubble generation regimes

MB production from the expanding-nozzle FFMD was divided into five regimes. The FFMD transitioned sequentially through these production regimes when operated at a fixed gas pressure and increasing liquid flow rate^{42,58,59} (Table II) (Fig. 2).

The liquid flow rate ranges that defined the production regimes were determined over a range of gas pressures (Fig. 3). The location of the boundaries between these regimes changed with gas pressure. For example, the stable regime at 45.5 kPa spanned a liquid flow rate range of 23 $\mu\text{L}/\text{min}$ (i.e., stable MBs were produced at all flow rates between 41 and 64 $\mu\text{L}/\text{min}$). At 29 kPa, however, the stable regime only spanned a flow rate range of 2.5 $\mu\text{L}/\text{min}$. The boundary between regimes II and III was defined as the minimum liquid flow rate that enabled the generation of MBs with diameters less than the height of the channel ($20.3\text{ }\mu\text{m}$ in this case) and was

FIG. 2. MB diameter and production rate curves for a fixed gas pressure (41.4 kPa), and increasing liquid flow rate. (A) Regime I (Table II), (B) Regime II, (C) Regime III, (D) Regime IV, and (E) Regime V. Scale bar = $20\text{ }\mu\text{m}$ for all images.

TABLE II. FFMD production regimes.

Name	Characteristics
Regime I	No MB formation, gas streams continuously through nozzle. ¹⁹
Regime II (cylindrical regime)	MB diameter larger than the height of the channel resulting in cylindrical MB formation. ^{19,32}
Regime III (stable regime)	MB diameter smaller than height of channel resulting in production of spherical monodisperse MBs. ^{19,20,60} Peak MB production rate occurs within this regime.
Regime IV (unstable regime)	Production of spherical MBs in doublets, triplets, or multiplets (i.e., production of several MBs of different sizes in rapid succession). ^{19,34} Production rapidly decreases within this regime.
Regime V	No MB generation, liquid streams continuously through nozzle.

found to be linearly related to gas pressure ($R^2 = 0.93$). The boundary between regimes IV and V was defined as the maximum liquid flow rate that enabled MB production and was also found to be linearly related to gas pressure ($R^2 = 0.99$). The boundary between regimes III and IV (i.e., stable and unstable regimes) was defined as the liquid flow rate for a given gas pressure at which multiplets (e.g., doublets and triplets) began to be produced and was best fit by a cubic relationship (adjusted- $R^2 = 0.99$). Additional studies, however, are required to elucidate the physical mechanisms for these regime transitions and how they are related to the input parameters. Interestingly, the width of the stable regime increased and the unstable regime decreased with increasing pressure, suggesting that at higher pressures, the FFMD may transition directly from regime III to regime V. For all liquid flow rates and gas pressure combinations, peak MB production always occurred within the stable regime. In addition, the smallest monodisperse MBs produced always occurred at the transition between the stable and unstable regimes. Since the stable regime resulted in stable generation of monodisperse MBs, the FFMDs were characterized in this regime alone to ensure repeatability and maximize the production rate.

The stable regime (III) was further subdivided into geometry-controlled (Fig. 3(d)) and flow-controlled (Fig. 3(e)) regimes. In the geometry-controlled regime, the maximum width of the gas thread (i.e., the narrowest part of the gas cone) was restricted by the dimensions of the nozzle. When generating a MB, the tip of the gas cone penetrated the nozzle and appeared to block the nozzle completely in the width dimension. The MB formed downstream of the nozzle while the neck of the gas thread was squeezed inwards by the liquid phase upstream of the

FIG. 3. (A) MB generation pattern from an expanding-nozzle FFMD. Black dotted lines are the fitted boundary lines between production regimes (Table II). Only gas pressure and liquid flow rate parameters that resulted in spherical MBs were plotted. (B) Monodisperse MBs (Regime III). (C) Multiplet MBs (Regime IV). (D) Geometry-controlled regime: gas cone tip (black arrows) oscillates over a larger extent and included the nozzle. Gas pressure = 37.9 kPa; liquid flow rate = 45 $\mu\text{L}/\text{min}$; exposure = 200 ns; period = 700 ns. (E) Flow-controlled regime: Gas cone tip oscillates over a narrower extent and was limited to downstream of the nozzle. Gas pressure = 45.5 kPa; liquid flow rate = 61 $\mu\text{L}/\text{min}$; exposure = 200 ns; period = 300 ns. Scale bar = 15 μm for all photographs.

nozzle. Eventually, the MB would pinch-off and the tip of the gas cone would retract to upstream of the nozzle.³⁵ After MB pinch-off, a waiting time was observed during which the tip of gas cone moved back towards the nozzle prior to penetrating again. This geometry-controlled regime was observed at lower gas pressures ($P \leq 41.3$ kPa) or liquid flow rates ($Q_l \leq 58 \mu\text{l}/\text{min}$). At higher gas pressures ($P > 41.3$ kPa) and liquid flow rates ($Q_l > 58 \mu\text{l}/\text{min}$),²⁰ a flow-controlled regime was observed. In this regime, the gas thread width was less than the width of the nozzle, was located in the center of the nozzle, and stayed downstream of the nozzle. Unlike the geometry-controlled regime, the tip of the gas cone in the flow-controlled regime always remained downstream of the nozzle (i.e., no waiting time) and retracted over a smaller distance after each MB pinch-off event.

D. Spherical monodisperse microbubble generation modes

The MB generation cycle from an FFMD operating within the geometry-controlled regime was separated into two parts: “bubbling time” (τ_b) and “waiting time” (τ_w). Bubbling time was defined as the duration between when the tip of the gas cone entered the nozzle to MB pinch-off (Fig. 3(d), 0.0–6.3 μs). Waiting time was defined as the time needed for the tip of the gas cone to re-enter the nozzle after MB pinch-off (Fig. 3(d), 7.7–13.3 μs). Combining these characteristic times resulted in a relationship for MB production rate f_b

$$f_b = \frac{1}{\tau_b + \tau_w}. \quad (6)$$

Consistent with Garstecki *et al.*,^{32,35} τ_b was observed to decrease with increasing liquid flow rate ($\partial\tau_b/\partial Q_l < 0$), and be independent of gas pressure. Waiting time (τ_w), however, was found to depend on both liquid flow rate and gas pressure. At low gas pressures (e.g., $P \leq 37.9$ kPa), τ_w increased with increasing liquid flow rate ($\partial\tau_w/\partial Q_l > 0$) and decreased with increasing gas pressure ($\partial\tau_w/\partial P < 0$). Interestingly, at higher gas pressures (e.g., $P \geq 41.4$ kPa), τ_w decreased with both liquid flow rate and gas pressure ($\partial\tau_w/\partial Q_l < 0$ and $\partial\tau_w/\partial P < 0$).

Consequently, two MB generation modes, separated by a transition pressure ($P_{\text{transition}}$), were defined within the geometry-controlled regime. Generation mode one occurred at pressures less than $P_{\text{transition}}$. In this mode, τ_b decreased and τ_w increased with liquid flow rate. The net affect was a production rate that did not increase more than 25% over the range of input parameters tested (Fig. 4). At gas pressures greater than $P_{\text{transition}}$, both τ_b and τ_w decreased with increasing liquid flow rate and the FFMD transitioned into generation mode two. Within this mode, MB production rate increased with increasing liquid flow rates.

E. Spherical monodisperse microbubble production rate analysis

For the parameters tested, the FFMD was operated with Reynolds numbers between 50–100; Weber numbers between 5–30; and capillary numbers between 0.1–0.25 (Table I). Previous models, however, were developed for microfluidic devices operated at either high Reynolds and Weber numbers^{20,31} or at low Reynolds and capillary numbers.³⁵ Furthermore, the gas cone shape in the flow-controlled regime was similar to that observed by Gañán-Calvo *et al.*,²⁹ while the gas cone shape in the geometry-controlled regime was similar to that observed by Garstecki *et al.*,³⁵ suggesting that the expanding-nozzle FFMD may be a hybrid of those two models.

The bubbling time and waiting time were modeled after Garstecki *et al.*^{32,35} (Eq. (2)). This relationship was extended by introducing the parameters β and ε to account for any dependence of these characteristic times on gas pressure

$$\tau_b = \frac{\beta_b(P)}{Q_l} + \varepsilon_b(P), \quad (7)$$

$$\tau_w = \frac{\beta_w(P)}{Q_l} + \varepsilon_w(P), \quad (8)$$

FIG. 4. MB production rate as a function of liquid flow rate at different gas pressures. The black dotted line indicates the observed boundary ($P_{transition}$) between the two MB generation modes.

where the units of τ and Q_l are μs and $\mu l/min$, respectively, and β and ε were empirically determined. The effect of viscosity on these parameters was not determined and will need further investigation. High speed images of geometry-controlled MB production were used to calculate the waiting and bubbling times, which were then used to fit Eqs. (7) and (8). Due to limits in resolution, bubbling and waiting times could not be measured during flow-controlled MB production.

As noted previously, τ_b was observed to be linearly related to the reciprocal of liquid flow rate ($1/Q_l$) but independent of gas pressure across all flow rates and pressures tested— $R^2 = 0.88$ and $\beta_b(P) = \beta_b = 477$, and $\varepsilon_b(P) = \varepsilon_b = -6.25$. τ_w was also related to the inverse of liquid flow rate for a fixed gas pressure, but the slope (β_w) and intercept (ε_w) of this relationship was observed to be linearly related to gas pressure— $R^2 = 0.86$ and 0.92 , respectively, $\beta_w(P) = 105P + 4.43 \times 10^3$ and $\varepsilon_w(P) = -3.43P + 150$, where the unit of P is kPa. Additional studies are required to determine how channel geometry and input parameters may affect β and ε .

Combining Eqs. (7) and (8) with Eq. (6) results in a final expression for production rate as a function of gas pressure and liquid flow rate

$$f_b = \frac{Q_l}{\beta_b + \beta_w(P) + [\varepsilon_b + \varepsilon_w(P)]Q_l}, \quad (9)$$

where the units of f_b , Q_l , and P are 10^3 MB/s, $\mu l/min$, and kPa, respectively. $P_{transition}$ ($\partial f_b / \partial Q_l = 0$) was calculated to be 41.8 kPa, which was close to the observed transition that occurred between 37.9 and 41.4 kPa (Fig. 4). This relationship between liquid flow rate, gas pressure, and production rate model matched the actual production rate within the gas pressure range ($37.9 \text{ kPa} < P < 43.4 \text{ kPa}$)— $R^2 = 0.91$ with 89% of the predicted values within 15% of the measured production rate (Fig. 5). At higher pressures ($P = 45.5 \text{ kPa}$, Fig. 5 filled markers) and liquid flow rates, production transitioned into the flow-controlled regime (Fig. 5 square markers), and the production rate relationship (Eq. (9)) began to deviate and was found to no longer be valid. In addition, the relationship appears to underestimate the production rate at the lowest gas pressures tested (Fig. 5, dotted box), suggesting the possibility of an additional production regime.

F. Spherical monodisperse microbubble production diameter analysis

Similar to Garstecki *et al.*^{32,35} (Eq. (4)), the volume of the generated MB was observed to be proportional to the product of the bubbling time (τ_b) and the gas pressure (P) when the FFMD was operated within the geometry-controlled regime

$$V_b \propto P\tau_b. \quad (10)$$

Combining Eqs. (7) and (10)

$$d_b \propto \left[P \left(\frac{\beta_b}{Q_l} + \varepsilon_b \right) \right]^{\frac{1}{3}}, \quad (11)$$

results in a relationship for MB diameter as a function of gas pressure and liquid flow rate. This phenomenological relationship exhibited a good correlation with MB diameter— $R^2 = 0.91$ —for gas pressures between 20.7 and 45.5 kPa, encompassing both geometry and flow-controlled regimes (Fig. 6). The residuals across all diameters exhibited a normal distribution, and no significant pattern was observed at the smaller diameters (Fig. 6, inset), suggesting that this relationship between MB diameter, gas pressure, and liquid flow rate remains valid for clinically relevant MB diameters (i.e., diameters less than $10 \mu\text{m}$) as well.

G. Limitations

For both the production rate and MB diameter relationships, Eqs. (9) and (11), respectively, only a single channel geometry and nozzle width was investigated. As a result, additional experiments are necessary to determine the effect of channel geometry on these relationships. In the model by Gañán-Calvo *et al.*,^{29,31} Eq. (3), the diameter of the nozzle was found to affect MB diameter and was explicitly included as a scaling factor D . In the model by Garstecki *et al.*,^{32,35} Eq. (4), however, the nozzle width (w) is not explicitly included although it may be implicitly incorporated into the proportionality constant. Similarly, for the relationships developed here, the effect of nozzle diameter on production rate and MB diameter may also be implicitly incorporated into β , ε , and the proportionality constant in Eq. (11).

In addition, Eqs. (9) and (11) are only valid for FFMD production of MBs within the geometry-controlled regime. Relationships describing the transition between regimes will therefore need to be further investigated as they are important in determining the gas pressure and liquid flow rates over which the FFMD will operate within the geometry-controlled production

FIG. 5. (a) Comparison of measured MB production rate (f_b) and the calculated production rate from Eq. (9) for gas pressures between 20.7 and 45.5 kPa. Red line is 1:1 correspondence between predicted and measured. Circle markers indicate geometry controlled and square markers indicate flow-controlled MB production, respectively. The dotted box outlines MB production rates measured at pressures between 29 and 34.5 kPa. For production rates measured at pressures between 37.9 and 43.4 kPa, $R^2 = 0.91$ and 89% of the predicted values were within 15% of the measured values (red dotted line). At higher pressures and flow rates, production transitioned into the flow-controlled regime, and Eq. (9) was no longer valid. At the highest pressure tested ($P = 45.5 \text{ kPa}$, filled markers), the production rate was overestimated across both geometry-controlled and flow-controlled production regimes. Inset: Zoom of pressures from 37.9 to 45.5 kPa.

FIG. 6. d_b versus $[P(\beta_b/Q_l + \varepsilon_b)]^{1/3}$ for gas pressures ranging from 20.7 to 45.5 kPa. Black line is a linear fit ($R^2 = 0.91$). Inset: Residuals.

mode. Furthermore, we observed that the minimum MB diameter produced by an FFMD operating in the geometry-controlled regime was equal to the nozzle width. While smaller MBs could be produced, the FFMD had to transition into a flow-controlled mode for this to occur. This suggests that the nozzle width may affect the transition between production regimes and that further investigation is necessary.

We do not, however, expect channel height to significantly impact MB diameter and production. Changes in channel height would affect both the gas pressure and the liquid flow rate. Since, both MB diameter and production rate are related to the ratio of gas pressure and liquid flow, we expect these changes to cancel each other out. Similarly, previous work by Dietrich *et al.*⁴⁴ suggests that the angle of the liquid channel intersection with the gas channel has a negligible effect on MB volume.

IV. CONCLUSION

Expanding-nozzle FFMDs were found to transition through five distinct regimes while generating MBs, resulting in the generation of cylindrical, spherical monodisperse (stable), and spherical multidisperse (unstable) MBs. Within the stable regime, MB pinch-off was observed to be either geometry-controlled or flow-controlled. Furthermore, FFMD production was further observed to transition between two generation modes as a function of pressure, one that was independent of liquid flow rate and a second that was dependent. A relationship between liquid flow rate, gas pressure, and MB production rate was obtained by analyzing two characteristic times—bubbling time and waiting time—within the geometry-controlled regime. In addition, an updated relationship for MB diameter that is the product of gas pressure and bubbling time was developed.

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