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Real-time Control of Microbubble Diameter from a Flow Focusing Microfluidic Device

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***Abstract*—Microbubbles (MBs), generally of 1−4 μm diameter, are useful in contrast ultrasound imaging. MBs can also be used as a therapeutic agent for sonothrombolysis when combined with ultrasound and thrombolytic drugs. Recently, large MBs (10−20 μm diameter) produced by flow focusing microfluidic devices (FFMDs) have been demonstrated to exhibit enhanced bioeffects for sonothrombolysis applications. However, one remaining problem that limits the adoption of this technology is the lack of dynamic monitoring and control of MB diameter. In this study, we demonstrate the regulation of MB diameter in real-time by the incorporation of proportional-integral (PI) feedback control system into an FFMD with an integrated micro Coulter particle counter. With this controller, MB diameters were produced between 14 and 24 μm.**

***Index Terms*—Ultrasound, microbubbles, sonothrombolysis, microfluidics, micro Coulter particle counter, feedback control system**

I. Introduction

Deep vein thrombosis and pulmonary embolism (DVT/PE) affect an estimated 300,000 to 600,000 people annually in the US [1]. Sonothrombolysis, using ultrasound, MBs, and thrombolytic drugs, has been investigated as a potential therapeutic approach for DVT and PE. Clot lysis rate was improved by sonothrombolysis in both *in vitro* and *in vivo* studies [2]–[4]. A recent study demonstrated that large MBs (10−20 μm diameter) produced by a catheter-mounted flow focusing microfluidic device (FFMD) exhibited a 4-fold increase in *in vitro* clot lysis rate versus a clinical dose of recombinant tissue plasminogen activator (rtPA) alone [5]. However, to enable future clinical translation, there must be a means to remotely perform real-time control of MB diameter of the FFMD. To address this limitation, a method to measure MB diameter is required.

Previous studies in our laboratory demonstrated that an FFMD design with an integrated micro Coulter particle counter (μCPC) placed within the expanding nozzle can measure MB diameter between 8 and 20 μm and production rate up to 3.25×10^5 MB/s [6]. This design detected the impedance change when MBs pass through the electrodes, enabling the computation of MB diameter using the change in voltage output measured across a Wheatstone bridge. To control the MB diameter in real-time, a closed-loop control system in microfluidic device [7], [8] must be implemented to automatically adjust operational parameters.

In this study, a feedback control system was implemented in an FFMD fabricated with an integrated μCPC to monitor MB diameter *in situ*.

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II. Methods

A. Device fabrication and MB production

An FFMD was cast in polydimethylsiloxane from a custom SU-8 mold, as in [9]. Electrodes were fabricated using a standard lift-off technique [10], [11] and the substrate with electrodes was bonded to the FFMD using plasma-activated bonding approach.

MBs were produced inside the FFMD with a continuous phase and a dispersed nitrogen phase. The continuous phase was comprised of 4% (w/v) bovine serum albumin, 10% dextrose (w/v) in 0.9% saline. The continuous phase was provided with flow rates between 18−26 μL/min using a syringe pump (PhD 2000, Harvard Apparatus, Holliston, MA) and the nitrogen was provided with pressure between 9−15 psi (62-103 kPa) by an electronic regulator (PC-series, Alicat Scientific, Tuscon, AZ).

B. Signal processing

The newly produced MB passed across the detection region and introduced an impedance change between the electrodes. The impedance change was detected by a Wheatstone bridge and amplified by a differential amplifier (LM6171, Texas Instruments, Dallas, Tx) [6]. The bridge was excited by a 4 V_{pp}, 1 MHz sinusoidal signal. The modulated signal was demodulated by quadrature demodulation [10] using MATLAB (Mathworks, Natick, MA) processing. The maximum voltage of the demodulated signal for each MB were selected by a peak detection algorithm in MATLAB and averaged over the whole record sampled at 20 MHz for 32768 data points, denoted as $\bar{V}_{max}$.

Under the flow rates mentioned above, MB electrical signal $\bar{V}_{max}$ and simultaneous high-speed images were acquired. The optical diameters were fit by a cubic function of $\bar{V}_{max}$ which was used for calculation of electrical MB diameter.

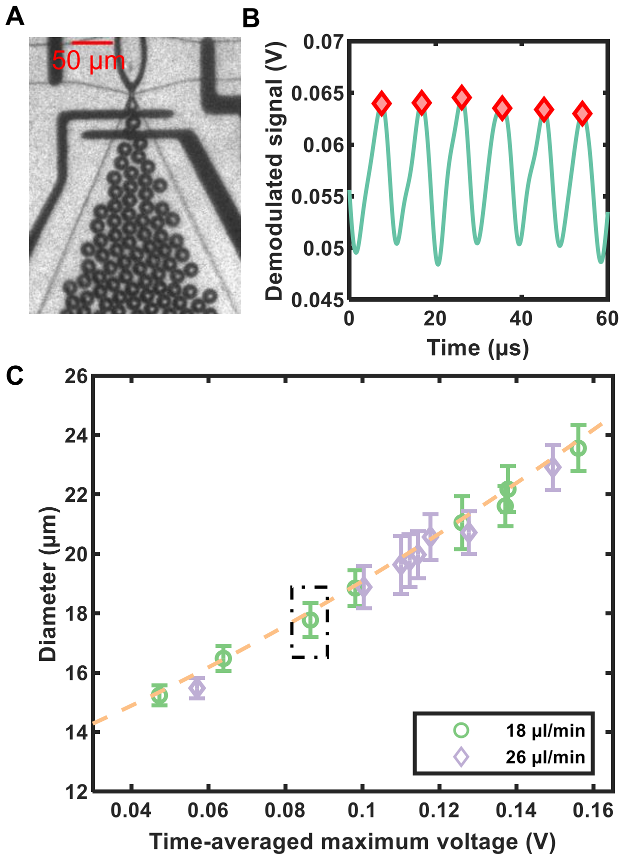

Fig. 1. (A) A benchtop FFMD with μCPC produces MBs of 17.8 μm. (B) Simultaneous demodulated signal is extracted from the modulated signal. The maxima marked by diamonds are selected and averaged over the sampling. (C) Diameters of MBs have a cubic function relation with time-averaged maximum voltage $\bar{V}_{max}$. Original data under two flow rates are plotted and the dashed line is the fitting result. The dash-dotted bounding box refers to the optical image in (A) and electrical signal in (B).

TABLE I
CHARACTERIZATION OF FEEDBACK CONTROL SYSTEM

Setpoint diameter (μm)	**20**	**22**	**24**
Rise time (s)	1.6	4.7	4.6
RMSE[a] in 15−36 s (μm)	0.065	0.13	0.21
Maximum error (μm)	0.23	0.46	0.55
Pressure change in 15−36 s (psi)	0.070	0.14	0.19

[a]Root mean squared error.

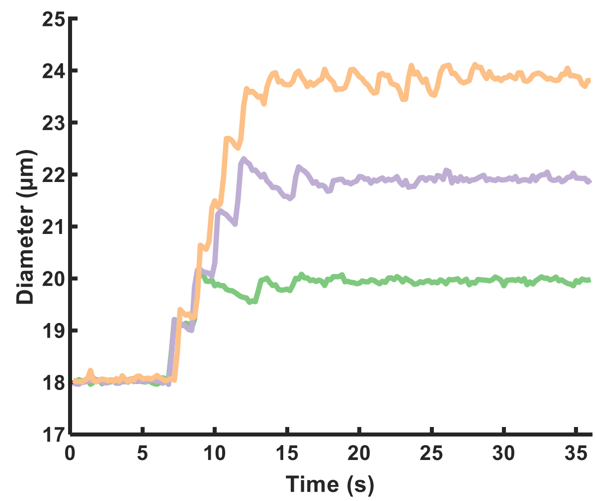

Fig. 2. Production of MBs was controlled by a PI controller from electrical diameter of 18 to 20, 22 and 24 μm. The solid lines are the measured diameters.

C. Feedback control system

During MB production, the electrical MB diameters were sampled. A real-time proportional-integral (PI) control system in LabVIEW (National Instruments, Austin, TX) was used to adjust the input gas pressure every 200 ms, enabling the production of MBs with desired setpoint diameter. A change in gas pressure resulted in an immediate change of MB diameter, which is superior to that achievable using liquid flow rate adjustment through long tubing.

III. RESULTS

MBs with diameter between 13 and 28 μm were produced by the FFMD with μCPC at production rates between 45×10^3 and 140×10^3 MB/s.

40 electrical signal and high speed camera images were acquired for validation, as presented in Fig. 1A and 1B. Each pulse in 1B represents a passage of a MB through the detection region and $\bar{V}_{max}$ was computed in MATLAB. Production rates of MBs were calculated by the time interval between two pulses and the electrical diameters $D_{electrical}$ by a fitting cubic function of $\bar{V}_{max}$ were compared to corresponding optical MB diameter. The root mean squared error (RMSE) between $D_{electrical}$ and the optically measured diameter was 0.43 μm, and the coefficient of determination, R^2, was 0.98 for MBs produced under 5 flow rates.

The electrical measurement of $D_{electrical}$ can be implemented in real-time. Therefore, a feedback control system was designed to monitor and regulate the MB diameter. Fig. 2 illustrated the step response of MB production from 18 to 20, 22 and 24 μm , respectively. The setpoint diameter affected the response in diameter, summarized in Table I. Once the diameter stabilized (e.g. between 15−36 s), the fluctuation and RMSE in measured diameter increased with greater setpoint diameter.

IV. DISCUSSION AND CONCLUSIONS

The time-averaged maximum voltage is a function of the MB diameter, which enables the real-time sizing of MB. This leads to the possibility of implementing a feedback control method to control MB diameter, even at high production rates.

The limits of the operational parameters have not yet been fully evaluated. However, when the MB diameter was large, the ripple in MB diameter and tendency of increasing pressure indicated that it required quick and precise regulation from electronic pressure regulator. It was also observed that the input pressure to maintain a certain MB diameter was not always constant, potentially limiting the production time of fixed-diameter MBs. To maintain MBs of 24 μm, assuming the start pressure is 12.5 psi, the lasting time can be estimated by pressure change in Table I to be approximately 300 s, avoiding an excess of regulator's adjustable range below 15 psi.

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REFERENCES

[1] M. G. Beckman, W. C. Hooper, S. E. Critchley, and T. L. Ortel, "Venous thromboembolism: a public health concern," *American Journal of Preventive Medicine*, vol. 38, no. 4 Suppl, pp. S495–501, 2010.

[2] N. Schleicher, A. J. Tomkins, M. Kampschulte, J.-M. Hyvelin, C. Botteron, M. Juenemann, M. Yeniguen, G. A. Krombach, M. Kaps, N. J. Spratt, T. Gerriets, and M. Nedelmann, "Sonothrombolysis with BR38 Microbubbles Improves Microvascular Patency in a Rat Model of Stroke," *PLOS ONE*, vol. 11, no. 4, p. e0152898, Apr. 2016.

[3] K. B. Bader, M. J. Gruber, and C. K. Holland, "Shaken and Stirred: Mechanisms of Ultrasound-Enhanced Thrombolysis," *Ultrasound in Medicine & Biology*, vol. 41, no. 1, pp. 187–196, Jan. 2015.

[4] A. J. Dixon, J. M. R. Rickel, B. D. Shin, A. L. Klibanov, and J. A. Hossack, "In Vitro Sonothrombolysis Enhancement by Transiently Stable Microbubbles Produced by a Flow-Focusing Microfluidic Device," *Annals of Biomedical Engineering*, vol. 46, no. 2, pp. 222–232, Feb. 2018.

[5] A. J. Dixon, J. Li, J.-M. R. Rickel, A. L. Klibanov, Z. Zuo, and J. A. Hossack, "Efficacy of Sonothrombolysis Using Microbubbles Produced by a Catheter-Based Microfluidic Device in a Rat Model of Ischemic Stroke," *Annals of Biomedical Engineering*, vol. 47, no. 4, pp. 1012–1022, Apr. 2019.

[6] J. M. R. Rickel, A. J. Dixon, A. L. Klibanov, and J. A. Hossack, "A flow focusing microfluidic device with an integrated Coulter particle counter for production, counting and size characterization of monodisperse microbubbles," *Lab on a Chip*, vol. 18, no. 17, pp. 2653–2664, Aug. 2018.

[7] E. Miller, M. Rotea, and J. P. Rothstein, "Microfluidic device incorporating closed loop feedback control for uniform and tunable production of micro-droplets," *Lab on a Chip*, vol. 10, no. 10, pp. 1293–1301, May 2010.

[8] W. Zeng, S. Li, and Z. Wang, "Closed-loop feedback control of droplet formation in a T-junction microdroplet generator," *Sensors and Actuators A: Physical*, vol. 233, pp. 542–547, Sep. 2015.

[9] A. H. Dhanaliwala, J. L. Chen, S. Wang, and J. A. Hossack, "Liquid flooded flow-focusing microfluidic device for in situ generation of monodisperse microbubbles," *Microfluidics and Nanofluidics*, vol. 14, no. 3, pp. 457–467, Mar. 2013.

[10] S. Gawad, L. Schild, and P. Renaud, "Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing," *Lab on a Chip*, vol. 1, no. 1, pp. 76–82, 2001.

[11] A. V. Jagtiani, J. Carletta, and J. Zhe, "An impedimetric approach for accurate particle sizing using a microfluidic Coulter counter," *Journal of Micromechanics and Microengineering*, vol. 21, no. 4, p. 045036, Mar. 2011.