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Production of standardized air bubbles: Application to embolism studies

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Gaseous emboli may arise and enter into the circulation system during various clinical procedures. In order to better understand their immediate and long-term consequences, clinical investigations following the insertion of air bubbles into the body as well as new detection techniques need to be carried on. To this end, a device has been developed to generate a uniform stream of microbubbles with diameters ranging between 20 and 300 μm. This device comprises a glass micropipette connected to an air pressure source. The micropipette tip crosses a variable liquid flow and the bubbles produced are carried away by the flow. These bubbles have a very narrow size and density distribution: 90% of the bubbles lie within ±6% of the mean radius and the number of bubbles does not vary more than 10%. The size and density of the produced bubbles can be controlled by adjusting three independent parameters: the liquid flow, the gas pressure level, and the micropipette shape. For a given micropipette, increasing the liquid flow or decreasing the gas pressure level leads to a reduction of bubble size while the number of bubbles produced increases. As an example, doubling of the liquid flow results in a variation in bubble size up to 40%. This technique offers the advantage of generating uniform bubbles of known size and number depending on the settings selected. It appears to be a valuable tool for embolism studies such as the development of ultrasonic methods for detection of gaseous emboli.
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I. INTRODUCTION

The occurrence of gas bubbles in intravascular and intracerebral circulation and its neurological or arterial repercussion are of great concern in several clinical procedures. Emboli have been detected following decompression,[1–3] insertion of prosthetic valves,[4,5] atrial fibrillation,[6] acute stroke,[7] and during carotid[8,9] or cardiac surgery.[10] Depending on the clinical situation, the nature of the emboli (gaseous or solid) and the number of embolic events can vary greatly. Hills and Butler[11] measured intravascular gaseous emboli ranging from 19 up to 700 μm following decompression in living dogs. Gersh[12] detected bubble sizes between 60 and 300 μm in both intravascular and extravascular sites. The number of embolic signals can also show wide variations. Georgiadis *et al.*[4] recorded between 0 and 620 embolic events per 30 min period for patients with prosthetic heart valves. Müllges *et al.*[13] have observed between 0.53 and 59.05 embolic signals per minute during extracorporeal circulation in patients undergoing cardiac surgery. According to these results, the development of a device that allows the production of air bubbles of controllable size and density would be a valuable tool for embolism studies, either for clinical (consequences following the insertion of gas bubbles into the circulation) or technical (new ways for detection and characterization of microemboli) investigations.

Various methods have been proposed to generate gas bubbles. One approach is based on electrolysis. Although this technique allows the production of very small bubbles (Miller[14] was able to generate bubbles between 2 and 15 μm), the wide size distribution and the high number of bubbles significantly limit this method of production. Furthermore, the production of the bubbles is not easy to reproduce. A second approach is based on the gas injection principle and it has been extensively studied theoretically and experimentally using different apparatuses.[15–22] Hills and Butler[23] have developed a method to produce a stream of microbubbles with narrow size distribution. Nitrogen was forced to pass through hypodermic needles to form bubbles. The bubbles produced ranged from 20 to 250 μm. More recently, following a similar principle, Gañan-Calvo and Gordillo[24] have described a nonlinear phenomenon based on microfluidic physics that allowed the production of air bubbles. Bubbles as small as 5 μm were created using different physical parameters such as the diameter of the orifice, the liquid viscosity, and surface tension. These methods offer the operator the possibility of producing a wide range of controllable bubble sizes but the number of bubbles pro-

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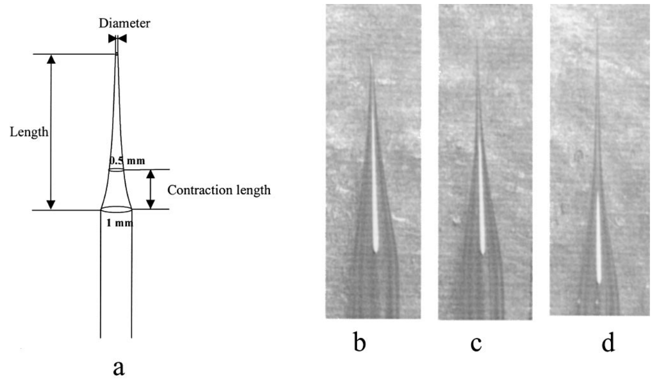


FIG. 1. (a) Definitions of the micropipette dimensions; (b)–(d) shape of micropipette types I, II, and III.

duced remains quite high and cannot be adjusted by the operator.

This article presents a method by which we generate a stream of bubbles of known size and number. A homemade micropipette is submerged into flowing liquid in a vertical tube. Gas is injected through the micropipette, allowing bubbles to escape from the micropipette tip and be transported by the liquid flow. The gas pressure, micropipette shape, and liquid flow can be adjusted to selectively produce bubble sizes ranging from 20 up to 300 μm.

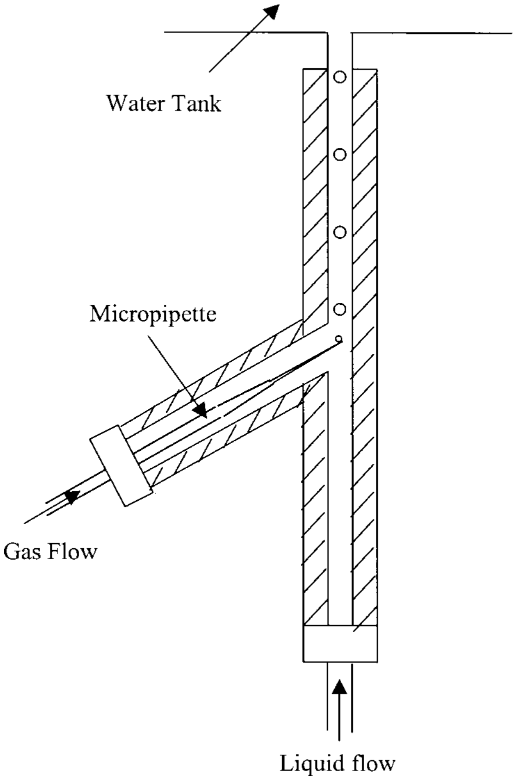


FIG. 2. Drawing of the bubble maker.

II. METHODS

A. Bubble generator

The micropipettes were made of glass capillaries (model GD 1, Narishige, Japan) with an outer diameter of 1 mm and an inner diameter of 0.6 mm. These tubes were placed in a pipette puller (Narishige, Japan). This instrument is designed to manufacture a variety of micropipettes through manual adjustment: the heating range and the weight, which is placed at the tip the tube. The variation of one of these parameters influences the shape and the diameter of the micropipette. Three parameters were measured: the inner diameter, the length, and the contraction length in order to specify the micropipettes. Their definitions are given in Fig. 1(a).

Several trials have been performed to demonstrate the effect of the puller settings on the produced micropipette: an increase of the heater level resulted in an increase of the micropipette length while its diameter and its contraction length decreased. When the weight attached at the end of the micropipette increased, the length and the contraction length increased while the diameter decreased. Examples of the micropipette are displayed in Figs. 1(b)–1(d) for three different puller settings: heater 65/weight 1 (type I), heater 70/weight 4 (type II), and heater 80/weight 1 (type III). For each case, three micropipettes were made and their dimensions measured. The variations in dimension are not greater than 3% for the micropipette length or 2% for the diameter while the contraction length remains identical. The pipette puller assures a reproducible way to generate micropipettes of known dimensions.

A custom-made bubble generator was developed based on the gas injection principle. Figure 2 shows a drawing of the bubble maker. This device is composed of a Y-shaped Plexiglas tube. The main channel is oriented vertically and has an internal diameter of 0.75 mm. The second channel is oriented at an angle of 26° vertically and has an inner diameter of 1.6 mm. A custom-made micropipette is positioned inside the second tube and its tip positioned in the center of the main channel. A variable gas pressure source is connected to an electromechanical valve that controls the gas injection rate. Flowing gas passes through the micropipette and generates a bubble at the tip. Nonpulsative liquid flow generated by a gear pump crosses the micropipette tip and carries the bubbles towards a water tank. For the current study, air was used and purified water was the surrounding liquid. To define the characteristics of the bubble patterns generated with the ‘‘bubble maker,’’ the bubble diameter and the bubble density number (expressed as the number of bubbles generated over a distance of 1 mm) were optically measured. The optical system is composed of a charge coupled device (CCD) camera (SONY) mounted on a zoom microscope (Sciencescope). Several frames were saved on a PC for further analysis. The diameter of the bubble and the density number were computed using a MATLAB program (Matlab, Mathworks). The diameters of 30 bubbles were measured as well as the distance separating two bubbles. The optical system was positioned 7 cm above the micropipette tip. Since the main application of interest is to direct the bubbles towards an ultrasound device, we do not measure the bubble diameters when they are released from the tip of the micropipette. The size measurements were made so as to notice any possible difference in bubble diameter at different locations in the water tank. However, no significant change was observed which can be explained by the fact that the

TABLE I. Dimensions of micropipette types I, II, and III.

Micropipette type	Length (mm)	Curvature (mm)	Diameter (μm)
I	4.7	1.7	3.17
II	4.38	1.91	2.4
III	6.7	1.7	1

time delay between the moment when the bubbles are created at the micropipette tip and the location where the bubbles are observed is short enough to keep the bubble diameter the same (dissolution effects are negligible). Measurement of the bubble number density was carried out instead of the frequency production of the bubbles commonly used. This choice was motivated by the ultrasonic application. Here, studies dealing with the interaction between bubbles and an ultrasound field require prior knowledge of the number of bubbles present in the ultrasound beam.

III. RESULTS

A. Bubble pattern characteristics

The pipette puller offers the possibility of creating a wide range of micropipettes. For this study, only three specific micropipettes were selected for further investigation of the parameters that affect the bubble characteristics. In Sec. III B, some general comments will be made concerning kinds of micropipettes and more specific remarks regarding the three micropipettes selected. These micropipettes are defined by settings of the pipette puller [heater (weight)]: type I: 65(1); type II: 70(4); type III: 80(1); see Fig. 1 and Table I.

The technique proposed resulted in the formation of a stream of microbubbles highly uniform in size and spacing. Depending on the type of micropipette, the bubble pattern (diameter and density number) characteristics strongly differ. Figures 3(a)–3(c) illustrate the different populations of bubbles that could be generated using micropipette types I, II, and III, using a liquid flow of 400 ml/h.

The size distributions are displayed in Fig. 4 for the three types of micropipettes at a selected liquid flow of 400 ml/h. The uniformity of the bubble diameter can be appreciated in Figs. 4(a)–4(c) and it appears to be dependent on the size of the bubbles produced: generation of the smallest bubbles [Fig. 4(a)] results in a very narrow size distribution: 100% of the bubbles lie within ±4 μm and 86% within ±2 μm. The bubbles produced by micropipette type I are much larger and their size distribution is somewhat wider: only 78% of the bubbles are less than ±4 μm and 70% less ±2 μm. The bubble's density number also exhibits a narrow distribution. Micropipettes types I, II, and III produce, respectively, maximal density number variations of 8.2%, 6.7%, and 12.3%. Overall, at any given flow and for any kind of micropipette, 90% of the bubbles have a diameter less than ±6% of their mean diameter while their density number does not exceed 10%.

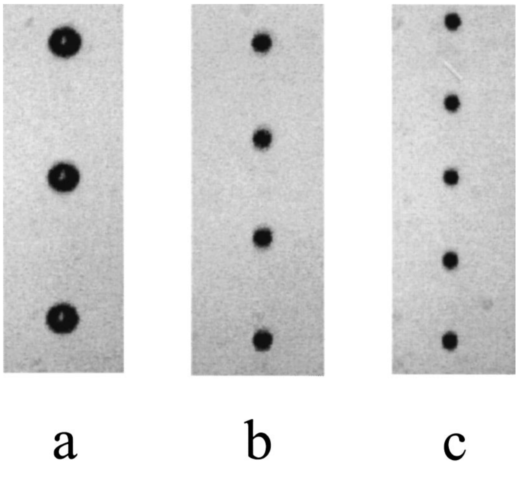


FIG. 3. Examples of bubbles produced by three selected micropipettes.

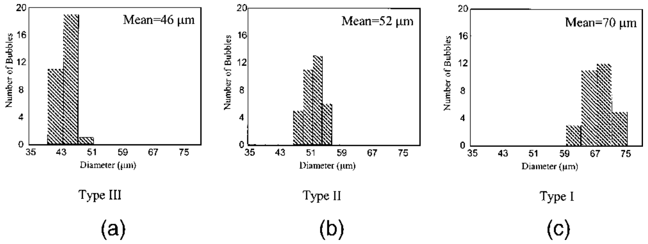


FIG. 4. Bubble size distributions for three types of micropipettes: (a) type I, (b) type II, and (c) type III.

To test the reproducibility of the bubble generation process, we performed three different sets of experiments: (a) the bubble maker was run for 30 min and the size of the bubbles was checked every 5 min; (b) the system was stopped and run again every hour for a period of 4 h. The measurements showed that the size and density distributions remain identical for these two sets of measurements. The last experiment (c) consisted of comparing the bubbles obtained from different micropipettes produced with the same pipette puller settings. As demonstrated in Sec. II, the variation in the micropipette dimension was small when the same settings of pipette puller were used. The bubble patterns for this configuration remained very similar. The mean diameter of the bubbles varied by 2 μm. The bubble density number could reach a difference of 1.3 bubbles/mm.

B. Parameters affecting the bubbles

The incidence of different parameters on the bubble size and number was investigated experimentally. The physics behind the bubble formation process have not been studied since the main objective of this work was to generate bubbles and explore the capability of the bubble maker for immediate application to gaseous emboli detection.

C. Gas pressure

The release of microbubbles at the micropipette tip requires the use of a minimal gas pressure level. This threshold depends on the dimensions of the micropipette employed. For micropipette types I, II, and III, the minimal gas pressure required is 2.3, 4, and 5 bar, respectively. Other examples of pressure threshold are displayed in Table II. The gas source could deliver pressures between 1 and 7 bar. In some cases,

TABLE II. Bubble diameter and number and minimal gas pressure levels for eight different micropipettes.

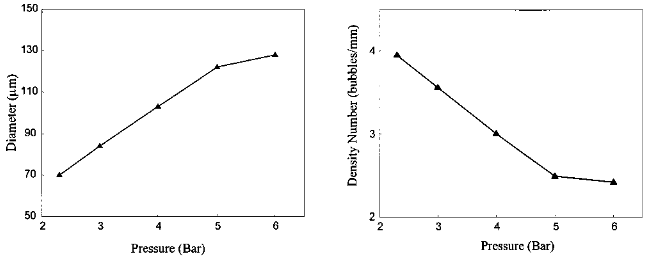
Heater	Weight	Pressure (bar)	Liquid flow (ml/h)	Bubble size (μm)	Density number (bubbles/mm)
80	1	5	110–640	112–20	1.9–15.8
80	2	6.5	160–600	91–30	2.94–11
75	2	5	120–400	140–52	0.7–3.4
70	1	3	160–400	152–59	1.3–4.5
70	4	4	120–640	140–30	0.95–9.25
65	1	2.3	160–400	226–70	0.85–2.83
65	4	2.8	160–400	162–61	1.5–5.2

higher pressures were required. So the micropipettes which required higher pressure threshold were discarded from this study.

The effects of the gas pressure on the bubble diameter were carried out by progressively increasing the gas pressure applied to the micropipette from 2.3 up to 6 bar. Micropipette type I was chosen since it has the lowest pressure threshold for bubble generation. Figure 5(a) presents the variation in bubble size as a function of the gas pressure for liquid flow of 400 ml/h. For pressures of 2.3 up to 5 bar, the bubble diameter increases linearly with the gas pressure. Typically, an increase of 20 μm is observed for a variation of 1 bar. At 6 bar, the relationship between the bubble size and the gas pressure is no longer linear. For pressures above 5 bar, the bubble size only increases very slightly. For higher pressures, the bubbles generated are nonuniform in size. The gas pressure is then too high for a stable rate of production.

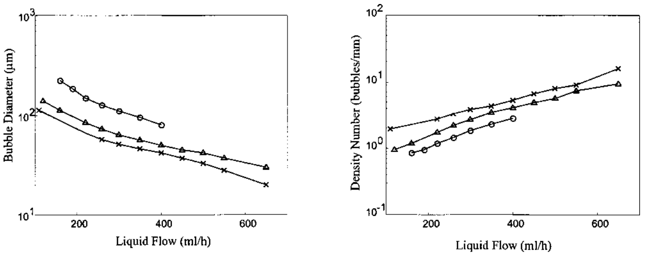
The influence of gas pressure on variation of the density number is displayed in Fig. 5(b). The density number decreases linearly with the gas pressure up to 5 bar. A decrease of around 0.6 bubbles/mm was measured for variation of 1 bar in the gas pressure. For pressures higher than 5 bar, the density of the bubbles remains almost identical. A high level of nonuniformity in the density of the bubbles was also noticed for higher pressures.

In order to quantify the change in bubble pattern with the gas pressure, micropipette types II and III were also studied. For both micropipettes and at a given liquid flow of 400 ml/h, the gas pressure was raised by 1.5 bar from the threshold of each micropipette. The variations in diameter and density number were measured following an increase in gas pressure. From these measurements it appears that variation of either the bubble diameter or the density number is strongly dependent on the type of micropipette and cannot be theoretically predicted. Micropipette type II did yield to an increase of 9 μm in diameter while micropipette types I and III, respectively, showed increases of 28 and 12 μm. The variations in bubble density number also depend on the type of micropipette. When the pressure is increased by 1.5 bar, the density number is reduced, respectively, by 0.9, 0.3, and 1.07 bubbles/mm for micropipette types I, II, and III. The changes in bubble pattern (size and density) relative to the gas pressure cannot be predicted independently for the kind of micropipette used.



(a) (b)

FIG. 5. (a) Variations in bubble size as a function of the air pressure. (b) Variations in bubble density number as a function of the air pressure.



(a) (b)

FIG. 6. (a) Variations in bubble size as a function of the liquid flow for three micropipettes: —○— type I, —△— type II, and —×— type III; (b) bubble density number as a function of the liquid flow of three micropipettes: —○— type I, —△— type II, and —×— type III.

In the current experimental setup, if the operator wants to select pressure as the main parameter by which to control the bubble diameter, the effects will be dominant for micropipettes that require a low gas pressure threshold to generate bubbles. Thus, the gas pressure can be progressively increased up to a level at which disturbances start appearing.

D. Liquid flow

The start of bubble generation occurs when the liquid flow is activated. For liquid flow smaller than 100 ml/h, the bubble diameter and density number tend to vary significantly. Bubble size varying by more than 30% has been observed. This effect may be explained by the fact that the liquid flow is not high enough to generate a stable rate of production. When the flow increases above this threshold the generation of bubbles is stabilized and is characterized by a clear stream of bubbles (Fig. 3). For high flow rates, the bubble size and density become progressively less uniform until the appearance of a cloud of bubbles. This upper limit of the liquid flow rate varies greatly depending on the micropipette used. As an example, micropipette type I does not produce uniform bubbles when the liquid flow is higher than 450 ml/h whereas for types II and III the liquid flow can be increased up to 800 and 1100 ml/h, respectively.

To study the effects of liquid flow on the bubble size and density number, measurements were performed with the liquid flow rate varying from 150 to 650 ml/h. For micropipette type I, the liquid flow was kept below 400 ml/h. Figure 6(a) shows on a semilogarithmic scale the variation of the bubble diameter as a function of liquid flow for the three types of micropipettes studied. As we can observe, the bubble size decreases when the liquid flow becomes higher. This relationship can be approximated by an exponential curve independent of the type of micropipette. The best fit was obtained using

$$\phi(f) = A1\,\exp(-f/350), \tag{1}$$

where ϕ is the bubble diameter in μm, f is the flow in ml/h, and $A1$ is a constant in μm that depends on the type of micropipette used. For micropipette types I, II, and III, coefficient $A1$ is, respectively, 260, 170, and 130 μm.

With a single micropipette, a wide range of bubbles can be generated by increasing only the liquid flow passing the micropipette tip. Micropipette types I, II, and III, respectively, produce bubbles between 20 and 112, 45 and 115, and 70 and 226 μm. The exponential curve indicates that small variations in the liquid flow rate induce large variations in the size of the bubbles produced. For example, an increase of the flow rate from 160 to 220 ml/h causes a change in bubble size of 17%.

The change in bubble density number as a function of liquid flow is displayed in Fig. 6(b) on a semilogarithmic scale. The bubble density number shows an exponential increase when the liquid flow increases. This also means that the frequency of production of bubbles increases with the liquid flow. Variation in the number of bubbles as a function of the liquid flow is independent of the type of micropipette used and can be approximated by

$$d(f) = A2\,\exp(f/250), \tag{2}$$

where d is the distance between two successive bubbles, f is the flow in ml/h, and $A2$ is constant depending on the micropipette used. For micropipette types I, II, and III, the coefficient $A2$ is, respectively, 0.5, 0.8, and 1.1.

As the liquid flow increases, the density of the bubbles becomes higher. This property offers the possibility of generating bubbles of fixed diameter but at a different rate. Micropipette type III generates bubbles of 100 μm with a density number of 2.5 bubbles/mm. Micropipette type II can generate bubbles of the same size with a density number of 1.6 bubbles/mm. By selecting adequate micropipettes, the bubble density can be controlled independently of the bubble diameter.

For liquid flow higher than 650 ml/h, the empirical curves indicate that bubbles with diameters less than 20 μm are expected. However, for such flows, the optical system does not allow accurate measurement of the bubble size and density due to blurring motion. According to our estimates, bubbles as small as 10 μm could be produced by this method using micropipette type III.

The influence of the gas pressure level on bubble pattern evolution with the liquid rate was also investigated. Figures 7(a) and 7(b) display the variation in bubble size and their separation versus the liquid flow for two different pressures $P1 = 5$ bar and $P2 = 6.5$ bar. The micropipette used here is type III. Both quantities decrease with the liquid flow according to the same exponential curve defined previously: Eq. (1) for the bubble diameter and Eq. (2) for the density number. Thus, the production of bubbles as a function of liquid flow is independent of the gas pressure level used.

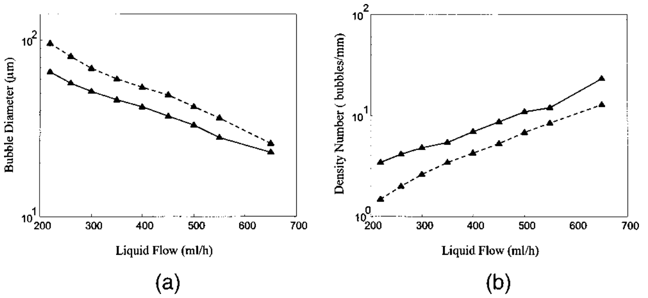


FIG. 7. (a) Variations in bubble diameter for two pressures: solid line is for 5 bar and dotted line is for 6.5 bar. (b) Variations in separation for two pressures: solid line is for 5 bar and dotted lines for 6.5 bar.

Based on the results presented here in Sec. III D, the size and density of bubbles as a function of liquid flow can be predicted using Eqs. (1) and (2) independently of the type of micropipette. The two constants, $A1$ and $A2$, have to be determined by performing a single measurement at any given flow.

E. Dimension of the micropipette

The third parameter that influences bubble production is the type of micropipette. Table II summarizes the results obtained for seven different micropipette configurations. Each micropipette is defined by the pipette puller settings (heater and weight). The minimal and maximal liquid flow rates used to generate bubbles are specified for each micropipette. Depending on the dimensions of the micropipette, the gas pressure required to generate bubbles and the range of bubble sizes produced vary significantly. The size of the bubbles is directly correlated to the diameter of the micropipette: the larger the diameter, the larger the bubbles. However, the gas pressure applied to the micropipette is also an important parameter. It was demonstrated in Sec. III D that the size of the bubble increases with the gas pressure applied and the gas pressure required to generate bubbles depends on the shape of the micropipette. Micropipettes with the smallest diameters do not necessary generate the smallest bubble size. As an example, we can compare the results obtained for micropipette type III with a new micropipette referred to as micropipette type IV. The latter micropipette was obtained at a heater level of 80 and two weights. Its dimensions are length 5.9 mm, curvature 1.5 mm, and diameter 0.8 μm. For these two micropipettes, the minimal gas pressures required to generate bubbles are, respectively, 5 and 6.5 bar. Even though micropipette type IV has a smaller diameter than micropipette type III, it appears that the bubbles generated by the two pipettes are almost identical. At a flow rate of 400 ml/h, micropipette type IV produced bubbles of 44 μm diameter while micropipette type III yielded bubbles of 42 μm. This example illustrates a case in which the effect of gas pressure on the bubble diameter cannot be compensated for by a smaller tip.

The density of the bubbles produced cannot be assessed with regard to the type of micropipette used. It was only observed that larger diameters produced less dense populations, but no quantitative information could be extracted from the measurements. We considered the case of micropipette type III and micropipette type IV. At a liquid flow rate of 400 ml/h, they produce almost identical bubble diameters but the number of bubbles differs. The density number is 7.4 bubbles/mm for micropipette type IV and 5.2 bubbles/mm for micropipette type III. Therefore, the shape of the micropipette can strongly affect the number of bubbles produced while there is negligible effect on the bubble diameters.

From Table II, we can appreciate the different bubbles' patterns. The diameters range from 20 up to 226 μm. Different micropipettes can produce the same bubble diameter at different liquid flows. If bubbles of 100 μm are required, five micropipettes can be selected: 75(2), 70(4), 70(1), 65(1), and 65(4). A difference will appear for the level of bubble density. For these five micropipettes, the average density number is, respectively, 1.1, 1.4, 1.6, 2.2, and 2.3 bubbles/mm.

IV. DISCUSSION

This article presents a system to generate calibrated microbubbles of highly uniform size and density number distribution. The bubble size and its number can be individually controlled by varying different settings. The bubble maker could be improved by studying other parameters that affect the bubble patterns such as the liquid's viscosity or surface tension. According to previous work, reducing the viscosity of the liquid leads to a decrease in bubble size that should allow the production of extremely small microbubbles. Using this setup, we have produced air bubbles of different sizes to simulate gaseous emboli that occur in blood circulation by applying ultrasound at different frequencies and amplitudes. We have concluded that the nonlinear behavior of these gas bubbles is more suitable as a detection parameter than the fundamental oscillations used in current emboli detection methods.[25]

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