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(54) Title: FORMATION OF PARTICLES FOR ULTRASOUND APPLICATION, DRUG RELEASE, AND OTHER USES, AND MICROFLUIDIC METHODS OF PREPARATION

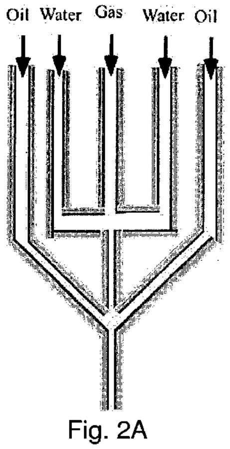

Fig. 2A

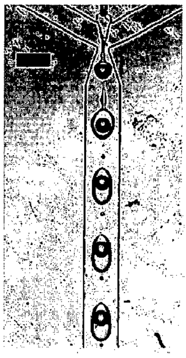

Fig. 2B

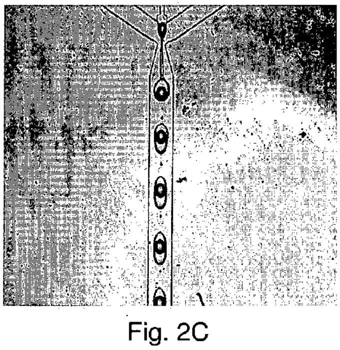

Fig. 2C

(57) **Abstract:** The present invention generally relates to emulsions and, in particular, to systems and methods for forming multiple emulsions and emulsions produced therefrom. A multiple emulsion generally describes a larger droplet that contains one or more smaller droplets therein which, in some cases, can contain even smaller droplets therein, etc. Multiple emulsions can be formed in certain embodiments with generally precise repeatability, and can be tailored to include any number of inner droplets, in any desired nesting arrangement, within a single outer droplet. In some cases, one (or more) of the fluids can be a gas. In addition, in some embodiments, the size of the multiple emulsion can be varied.

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FORMATION OF PARTICLES FOR ULTRASOUND APPLICATION, DRUG RELEASE, AND OTHER USES, AND MICROFLUIDIC METHODS OF PREPARATION

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/997,994, filed October 5, 2007, entitled "Formation of Particles for Ultrasound Application, Drug Release, and Other Uses, and Microfluidic Methods of Preparation," by Stone, *et al.,* incorporated herein by reference.

FIELD OF INVENTION

The present invention generally relates to emulsions and, in particular, to systems and methods for forming multiple emulsions and multiple emulsions produced therefrom.

BACKGROUND

An emulsion is a fluidic state which exists when a first fluid is dispersed in a second fluid that is typically immiscible or substantially immiscible with the first fluid. Examples of common emulsions are oil in water and water in oil emulsions. Multiple emulsions are emulsions that are formed with more than two fluids, or two or more fluids arranged in a more complex manner than a typical two-fluid emulsion. For example, a multiple emulsion may be oil-in-water-in-oil ("o/w/o"), or water-in-oil-in-water ("w/o/w"). Multiple emulsions are of particular interest because of current and potential applications in fields such as pharmaceutical delivery, paints and coatings, food and beverage, chemical separations, and health and beauty aids.

Typically, multiple emulsions of a droplet inside another droplet are made using a two-stage emulsification technique, such as by applying shear forces through mixing to reduce the size of droplets formed during the emulsification process. Other methods such as membrane emulsification techniques using, for example, a porous glass membrane, have also been used to produce water-in-oil-in-water emulsions. Microfluidic techniques have also been used to produce droplets inside of droplets using a procedure including two or more steps. For example, see International Patent

Application No. PCT/US2004/010903, filed April 9, 2004, entitled "Formation and Control of Fluidic Species," by Link, *et al.*, published as WO 2004/091763 on October 28, 2004; or International Patent Application No. PCT/US03/20542, filed June 30, 2003, entitled "Method and Apparatus for Fluid Dispersion," by Stone, *et al.*, published as WO 2004/002627 on January 8, 2004, each of which is incorporated herein by reference. See also Anna, *et al.*, "Formation of Dispersions using 'Flow Focusing' in Microchannels," *Appl. Phys. Lett.*, 82:364 (2003) and Okushima, *et al.*, "Controlled Production of Monodispersed Emulsions by Two-Step Droplet Breakup in Microfluidic Devices," *Langmuir* 20:9905-9908 (2004). In some of these examples, a T-shaped junction in a microfluidic device is used to first form an aqueous droplet in an oil phase, which is then carried downstream to another T-junction where the aqueous droplet contained in the oil phase is introduced into another aqueous phase. In another technique, co-axial jets can be used to produce coated droplets, but these coated droplets must be re-emulsified into the continuous phase in order to form a multiple emulsion. See Loscertales *et al.*, "Micro/Nano Encapsulation via Electrified Coaxial Liquid Jets," *Science* 295:1695 (2002).

Multiple emulsions and the products that can be made from them can be used to produce a variety of products useful in the food, coatings, cosmetic, chemical, or pharmaceutical industries, for example.

SUMMARY OF THE INVENTION

The present invention generally relates to emulsions and, in particular, to systems and methods for forming multiple emulsions and emulsions produced therefrom. The subject matter of the present invention involves, in some cases, interrelated products, alternative solutions to a particular problem, and/or a plurality of different uses of one or more systems and/or articles.

In one aspect, the invention is directed to a method. One set of embodiments comprises acts of providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third, liquid fluid, with at least one of the first and second fluids being a gas, and altering the volume of the gas by applying ultrasound to at least a portion of the gas.

Another set of embodiments comprises acts of providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third, liquid fluid, wherein at least one of the first fluid and the second fluid is a gas, and altering the volume of the gas by at least about 5 percent.

Yet another set of embodiments comprises acts of providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third, liquid fluid, and causing at least one of the first fluid and the second fluid to rupture.

Another set of embodiments comprises acts of administering, to a subject, a multiple emulsion having at least two nested fluids, and applying ultrasound to the subject.

Yet another set of embodiments comprises acts of administering, to a subject, a multiple emulsion having at least two nested fluids, and thereafter, rupturing at least one of the fluids contained within the multiple emulsion.

In another aspect, the present invention is directed to a method of making one or more of the embodiments described herein. In another aspect, the present invention is directed to a method of using one or more of the embodiments described herein.

Other advantages and novel features of the present invention will become apparent from the following detailed description of various non-limiting embodiments of the invention when considered in conjunction with the accompanying figures. In cases where the present specification and a document incorporated by reference include conflicting and/or inconsistent disclosure, the present specification shall control. If two or more documents incorporated by reference include conflicting and/or inconsistent disclosure with respect to each other, then the document having the later effective date shall control.

BRIEF DESCRIPTION OF THE DRAWINGS

Non-limiting embodiments of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale. In the figures, each identical or nearly identical component illustrated is typically represented by a single numeral. For purposes of clarity, not every component is labeled in every figure, nor is every component of each embodiment of the invention shown where illustration is not necessary to allow those of ordinary skill in the art to understand the invention. In the figures:

Figs. 1A-1C illustrate a method for making multiple emulsions according to one embodiment of the invention;

Figs. 2A-2C illustrate another method for making multiple emulsions according to another embodiment of the invention;

Figs. 3A-3G illustrate data indicating control over droplet formation according to one embodiment of the invention;

Figs. 4A-4G illustrate various multiple emulsions, produced using various embodiments of the invention;

Figs. 5A-5D illustrate data indicating control over droplet formation and nesting according to one embodiment of the invention;

Figs. 6A-6D illustrate schematic diagrams and images of experimental setups used to generate gas-in-water-in-oil emulsions, according to one set of embodiments;

Figs. 7A-7F illustrate images and a plot illustrating methods for controlling bubbles in a droplet, according to one set of embodiments;

Figs. 8A-8E illustrate experimental data and an image illustrating flow rate ratios on droplets and encapsulated bubbles, according to another set of embodiments; and

Figs. 9A-9D illustrate images and data relating to porous polyacrylamide microparticles, according to yet another set of embodiments.

DETAILED DESCRIPTION

The present invention generally relates to emulsions and, in particular, to systems and methods for forming multiple emulsions and emulsions produced therefrom. A multiple emulsion generally describes a larger droplet that contains one or more smaller droplets therein which, in some cases, can contain even smaller droplets therein, etc. Multiple emulsions can be formed in certain embodiments with generally precise repeatability, and can be tailored to include any number of inner droplets, in any desired nesting arrangement, within a single outer droplet. In some cases, one (or more) of the fluids can be a gas. In addition, in some embodiments, the size of the multiple emulsion can be varied.

Fields in which multiple emulsions may prove useful include (but are not limited to), for example, food, beverage, health and beauty aids, paints and coatings, chemical separations, and drugs and drug delivery. For instance, a precise quantity of a drug, pharmaceutical, or other agent can be encapsulated by a shell designed to release its contents under particular conditions, as described in detail below. Other species that can be stored (e.g., in the first fluid, second fluid, third fluid, etc. of the emulsion) and/or delivered include, for example, biochemical species such as nucleic acids such as RNA or DNA, proteins, peptides, or enzymes. Additional species that can be incorporated within a multiple emulsion of the invention include, but are not limited to, nanoparticles, quantum dots, fragrances, proteins, indicators, dyes, fluorescent species, chemicals, or the like.

Using the methods and devices described herein, in some embodiments, a consistent size and/or number of droplets can be produced, and/or a consistent ratio of size and/or number of outer droplets to inner droplets, inner droplets to other inner droplets, or other such ratios, can be produced. For example, in some cases, a single droplet within an outer droplet of predictable size can be used to provide a specific quantity of a drug. In addition, combinations of compounds or drugs may be stored, transported, and/or delivered in a multiple emulsion droplet. For instance, hydrophobic and hydrophilic species can be delivered in a single, multiple emulsion droplet, as the droplet can include both hydrophilic and hydrophobic portions. The amount and concentration of each of these portions can be consistently controlled according to certain embodiments of the invention, which can provide for a predictable and consistent ratio of two or more species in the multiple emulsion droplet.

Various aspects of the present invention are generally directed to multiple emulsions, which includes larger fluidic droplets that contain one or more smaller droplets therein which, in some cases, can contain even smaller droplets therein, etc. In some cases, the multiple emulsion is surrounded by a liquid (e.g., suspended). Any of these droplets may be of substantially the same shape and/or size (i.e., "monodisperse"), or of different shapes and/or sizes, depending on the particular application. As used herein, the term "fluid" generally refers to a substance that tends to flow and to conform to the outline of its container, i.e., a liquid, a gas, a viscoelastic fluid, etc. Typically, fluids are materials that are unable to withstand a static shear stress, and when a shear stress is applied, the fluid experiences a continuing and permanent distortion. The fluid may have any suitable viscosity that permits flow. If two or more fluids are present, each fluid may be independently selected among essentially any fluids (liquids, gases, and the like) by those of ordinary skill in the art, by considering the relationship between the fluids. In some cases, the droplets may be contained within a carrier fluid, e.g., a liquid.

A "droplet," as used herein, is an isolated portion of a first fluid that is surrounded by a second fluid. It is to be noted that a droplet is not necessarily spherical, but may assume other shapes as well, for example, depending on the external environment. In one set of embodiments, the droplet has a minimum cross-sectional dimension that is substantially equal to the largest dimension of the channel perpendicular to fluid flow in which the droplet is located.

In certain instances, the droplets may be contained within a carrying fluid, e.g., within a fluidic stream. The fluidic stream, in one set of embodiments, is created using a microfluidic system, discussed in detail below. In some cases, the droplets will have a homogenous distribution of diameters, i.e., the droplets may have a distribution of diameters such that no more than about 10%, about 5%, about 3%, about 1%, about 0.03%, or about 0.01% of the droplets have an average diameter greater than about 10%, about 5%, about 3%, about 1%, about 0.03%, or about 0.01% of the average diameter of the droplets. Techniques for producing such a homogenous distribution of diameters are disclosed in International Patent Application No. PCT/US2004/010903, filed April 9, 2004, entitled "Formation and Control of Fluidic Species," by Link, et al., published as WO 2004/091763 on October 28, 2004, incorporated herein by reference, and in other references as described below.

The fluidic droplets (in any nesting level) may each be substantially the same shape and/or size. Typically, monodisperse droplets are of substantially the same size. The shape and/or size of the fluidic droplets can be determined, for example, by measuring the average diameter or other characteristic dimension of the droplets. The "average diameter" of a plurality or series of droplets is the arithmetic average of the average diameters of each of the droplets. Those of ordinary skill in the art will be able to determine the average diameter (or other characteristic dimension) of a plurality or series of droplets, for example, using laser light scattering, microscopic examination, or other known techniques. The average diameter of a single droplet, in a non-spherical droplet, is the diameter of a perfect sphere having the same volume as the non-spherical droplet. The average diameter of a droplet (and/or of a plurality or series of droplets) may be, for example, less than about 1 mm, less than about 500 micrometers, less than about 200 micrometers, less than about 100 micrometers, less than about 75 micrometers, less than about 50 micrometers, less than about 25 micrometers, less than about 10 micrometers, or less than about 5 micrometers in some cases. The average diameter may also be at least about 1 micrometer, at least about 2 micrometers, at least about 3 micrometers, at least about 5 micrometers, at least about 10 micrometers, at least about 15 micrometers, or at least about 20 micrometers in certain cases.

The term “determining,” as used herein, generally refers to the analysis or measurement of a species, for example, quantitatively or qualitatively, and/or the detection of the presence or absence of the species. “Determining” may also refer to the analysis or measurement of an interaction between two or more species, for example, quantitatively or qualitatively, or by detecting the presence or absence of the interaction. Examples of suitable techniques include, but are not limited to, spectroscopy such as infrared, absorption, fluorescence, UV/visible, FTIR (“Fourier Transform Infrared Spectroscopy”), or Raman; gravimetric techniques; ellipsometry; piezoelectric measurements; immunoassays; electrochemical measurements; optical measurements such as optical density measurements; circular dichroism; light scattering measurements such as quasielectric light scattering; polarimetry; refractometry; or turbidity measurements.

One aspect of the present invention is generally directed to multiple emulsions, which includes larger fluidic droplets that contain one or more smaller droplets therein which, in some cases, can contain even smaller droplets therein, etc. Any number of nested fluids can be produced as discussed in detail below, and accordingly, additional third, fourth, fifth, sixth, etc. fluids may be added in some embodiments of the invention to produce increasingly complex droplets within droplets. For example, an outer fluidic droplet may contain one, two, three, four, or more first fluidic droplets (i.e., composed of a first fluid), some or all of which can contain one, two, three, four, or more second fluidic droplets (i.e., composed of a second fluid).

Some of these fluids may be the same, in certain embodiments (e.g., the second fluid may have the same composition as the outer fluid). There may be any number of nestings present. For example, the second fluidic droplets may contain one, two, three, four, or more third fluidic droplets; optionally, the third fluidic droplets may contain one, two, three, four, or more third fourth droplets, and so on. Within each nesting level (defined by one or more fluidic droplets each contained within a surrounding fluidic droplet), any number of fluidic droplets may be present, for example, for any given nesting level, one, two, three, four, or more fluidic droplets may be contained within a surrounding fluidic droplet. In addition, the number of the droplets in each nesting level may be controlled independently of the number of droplets in other nesting levels. In certain cases, any of these droplets may contain one or more species (e.g., molecules, particles, etc.), as described below. For example, the species may be contained within the innermost droplet(s) of a nesting of droplets.

In some cases, for a given nesting level, each of the fluidic droplets of that level may contain substantially the same number of inner fluidic droplets therein; for example, substantially all of the outer fluidic droplets may contain substantially the same number of first fluidic droplets, and/or substantially all of the first fluidic droplets may contain substantially the same number of second fluidic droplets therein, etc. It should be understood that, even if the droplets appear to be substantially identical, or to contain substantially the same number of droplets therein, not all of the droplets will necessarily be completely identical. In some cases, there may be minor variations in the number and/or size of droplets contained within a surrounding droplet. Thus, in some cases, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% of a plurality of outer droplets may each contain the same number of first fluidic droplets therein, and/or the same number of second fluidic droplets therein, etc. Similarly, in some embodiments, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% of a plurality of first droplets may each contain the same number of second droplets therein, etc.

In some embodiments, however, a plurality of outer droplets each may not necessarily contain substantially the same number of inner fluidic droplets therein, but each of the plurality of outer droplets contains two or more first fluidic droplets, some or all of which can contain second fluidic droplets (and optionally, third fluidic droplets nested within the second fluidic droplets, etc. For example, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 92%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% of a plurality of outer fluidic droplets may each contain more than two first fluidic droplets, and/or one or more second fluidic droplets, etc.

As a non-limiting example, in one set of embodiments, a triple emulsion may be produced, i.e., an emulsion containing outer fluid, containing droplets containing an outer fluid, some of which droplets can contain one or more inner fluidic droplets therein. In some cases, the carrying fluid and the inner fluid may be the same. The fluids in the triple emulsion are often of varying miscibilities, due to differences in hydrophobicity. For example, the carrying fluid may be water soluble (i.e., miscible in water), the outer fluid oil soluble (or immiscible in water), and the inner fluid water soluble. This arrangement is often referred to as a w/o/w multiple emulsion ("water/oil/water"). Another multiple emulsion may include a carrying fluid that is oil soluble (or immiscible in water), an outer fluid that is water soluble, and an inner fluid that is oil soluble. This type of multiple emulsion is often referred to as an o/w/o multiple emulsion ("oil/water/oil"). It should be noted that the term "oil" in the above terminology merely refers to a fluid that is generally more hydrophobic and not miscible in water, as is known in the art. Thus, the oil may be a hydrocarbon in some embodiments, but in other embodiments, the oil may comprise other hydrophobic fluids.

More specifically, as used herein, two fluids are immiscible, or not miscible, with each other when one is not soluble in the other to a level of at least 10% by weight at the temperature and under the conditions at which the multiple emulsion is produced. For instance, two fluids may be selected to be immiscible within the time frame of the formation of the fluidic droplets. In some embodiments, the carrying and inner fluids are compatible, or miscible, while the outer fluid is incompatible or immiscible with one or both of the carrying and inner fluids. In other embodiments, however, all three fluids may be mutually immiscible, and in certain cases, all of the fluids do not all necessarily have to be water soluble. In still other embodiments, as mentioned, additional fourth, fifth, sixth, etc. fluids may be added to produce increasingly complex droplets within droplets, e.g., an outer fluid may surround a first fluid, which may in turn surround a second fluid, which may in turn surround a third fluid, which in turn surround a fourth fluid, etc. In addition, the physical properties of each nesting layer of fluidic droplets may each be independently controlled, e.g., by control over the composition of each nesting level.

In some embodiments, the fluidic droplets, or at least a portion thereof (e.g., one or more fluids contained within the droplet, e.g., the core and/or a shell), may be solidified to form a solid. Any technique able to solidify a fluid can be used. For example, a fluid may be cooled to a temperature below the melting point or glass transition temperature of the fluid, a chemical reaction may be induced that causes the fluid to solidify (for example, a polymerization reaction, a reaction between two fluids that produces a solid product, etc.), or the like.

In one embodiment, the fluidic droplet (or portion thereof, such as an outer fluid) is solidified by reducing the temperature of the fluidic droplet to a temperature that causes at least one of the components of the fluidic droplet to reach a solid state. For example, the fluidic droplet may be solidified by cooling the fluidic droplet to a temperature that is below the melting point or glass transition temperature of a component of the fluidic droplet, thereby causing the fluidic droplet (or a portion thereof) to become solid. As non-limiting examples, the fluidic droplet may be formed at an elevated temperature (i.e., above room temperature, about 25 °C), then cooled, e.g., to room temperature or to a temperature below room temperature; the fluidic droplet may be formed at room temperature, then cooled to a temperature below room temperature, or the like.

In one embodiment, the fluidic droplet or portion thereof is solidified using a chemical and/or a polymerization reaction that causes solidification of a fluid to occur. For example, two or more fluids added to a fluidic droplet may react to produce a solid product, thereby causing formation of a solid particle. As another example, a first reactant within the fluidic droplet may be reacted with a second reactant within the liquid surrounding the fluidic droplet to produce a solid, which may thus coat the fluidic droplet within a solid “shell” in some cases, thereby forming a core/shell particle having a solid shell or exterior, and a fluidic core or interior, e.g., containing liquid or gas. As yet another example, a polymerization reaction may be initiated within a fluidic droplet, thereby causing the formation of a polymeric particle. For instance, the fluidic droplet may contain one or more monomer or oligomer precursors (e.g., dissolved and/or suspended within the fluidic droplet), which may polymerize to form a polymer that is solid. The polymerization reaction may occur spontaneously, or be initiated in some fashion, e.g., during formation of the fluidic droplet, or after the fluidic droplet has been formed. For instance, the polymerization reaction may be initiated by adding an initiator to the fluidic droplet, by applying light or other electromagnetic energy to the fluidic droplet (e.g., to initiate a photopolymerization reaction), or the like.

In some cases, the fluidic droplet may comprise a material having a sol state and a gel state (e.g., a hydrogel), such that the conversion of the material from the sol state into a gel state causes the fluidic droplet (or portion thereof, such as a shell) to solidify. The conversion of the sol state of the material within the fluidic droplet into a gel state may be accomplished through any technique known to those of ordinary skill in the art, for instance, by cooling the fluidic droplet, by initiating a polymeric reaction within the droplet, etc. For example, if the material includes agarose, the fluidic droplet containing the agarose may be produced at a temperature above the gelling temperature of agarose, then subsequently cooled, causing the agarose to enter a gel state. As another example, if the fluidic droplet contains acrylamide (e.g., dissolved within the fluidic droplet), the acrylamide may be polymerized (e.g., using tetramethylethylenediamine) to produce a polymeric particle comprising polyacrylamide, for example, as a hollow particle containing a fluid therein.

Another non-limiting example of a solidification reaction is a polymerization reaction involving production of a nylon (e.g., a polyamide), for example, from a diacid chloride and a diamine. Those of ordinary skill in the art will know of various suitable nylon-production techniques. For example, nylon-6,6 may be produced by reacting adipoyl chloride and 1,6-diaminohexane. For instance, a fluidic droplet may be solidified by reacting adipoyl chloride in the continuous phase with 1,6-diaminohexane within the fluidic droplet, which can react to form nylon-6,6 at the surface of the fluidic droplet. Depending on the reaction conditions, nylon-6,6 may be produced at the surface of the fluidic droplet (forming a particle having a solid exterior and a fluidic interior), or within the fluidic droplet (forming a solid particle).

Thus, in some cases, a porous particle can be produced. For example, a fluid within a multiple emulsion that contains a gaseous inner fluid may be hardened, thereby resulting in a hardened particle containing the inner gaseous fluid, rending the particle at least partially porous. Non-limiting examples of such particles are disclosed below in Example 3.

In one aspect, the invention is directed to a method, e.g., of manipulating a fluidic droplet, and/or a portion of the fluidic droplet, for example, a gas contained within the fluidic droplet. As examples, in some embodiments, a first fluid is surrounded by a second fluid and the second fluid is surrounded by a third, liquid fluid. In some cases, the first or second fluid comprises a gas. In other embodiments, the first fluid comprises a gas and the second fluid comprises a liquid. In other embodiments, the first fluid comprises a liquid and the second fluid comprises a gas. In some cases, the liquid may comprise water. In some instances, at least one of the first fluid and the second fluid may comprise air. In some embodiments, at least one of the first fluid and the second fluid may comprise nitrogen (N_2). In certain cases, the first or second fluid or the outermost liquid comprises a polymer, polymeric precursors, a gel, a hydrogel, polyacrylamide, or the like. In some cases, the fluids can contain a drug, e.g., as discussed below.

In some embodiments, the volume of the gas in the first or second fluid can be altered. The volume of the gas can be altered due to, for example, a chemical reaction, heating, a change in pressure, diffusion of a substance into or out of the gas, or the like. Other non-limiting examples of altering the volume of the gas (e.g., application of ultrasound) are included below. In some embodiments, the volume of gas can be altered by at least about 5 percent, at least 10 percent, at least 50 percent, at least 100 percent, at least 200 percent, or at least 500 percent, etc. The volume of the gas can be increased, for example, to release a substance from within the droplet containing the gas. The substance may be present within the gas, and/or present within another portion of the fluidic droplet. Non-limiting examples are discussed in detail below.

In some embodiments, the gas volume can be altered by heating the gas. In some cases, the gas can be heated to a temperature of at least about 30 °C, at least about 40 °C, at least about 50 °C, at least about 100 °C, at least about 200 °C, or at least about 500 °C, etc. Any suitable method may be used to heat the gas. For instance, in some embodiments, the gas can be heated using a localized heater. In another embodiment, the gas can be heated by causing a chemical reaction to occur that alters the volume of the gas. In other embodiments, the gas can be heated by exposing at least a portion of the gas to ultrasound or ultrasonic waves.

The volume of the gas, in one set of embodiments, can be altered by applying ultrasound to at least a portion of the gas. Any suitable system may be used to apply the ultrasound, e.g., a commercially available ultrasound generator. In some embodiments, the ultrasonic frequency used to alter the volume of the gas is at least about 20 kHz, at least about 500 kHz, at least about 1 MHz, or at least about 10 MHz, or the like. In some cases, more than one frequency of ultrasound may be applied, e.g., an ultrasound device may produce a range of ultrasound frequencies. In certain embodiments, the average frequency produced by the ultrasound device may have an average frequency of at least about 20 kHz, at least about 500 kHz, at least about 1 MHz, or at least about 10 MHz, or the like.

In some cases, altering the volume of the gas may comprise oscillating the volume of the gas, e.g., altering the volume in a cyclic pattern. Such oscillations may be useful, for instance, to heat the gas, or to improve detection of the gas, etc. In some embodiments, the volume of the gas can be oscillated at a frequency of at least about 1 Hz, at least about 10 Hz, at least about 100 Hz, at least about 1 kHz, at least about 10 kHz, at least about 100 kHz, at least about 1 MHz, at least about 10 MHz, at least about 1 GHz, or at least about 10 GHz, etc. The frequency of oscillation can be controlled by the frequency of the ultrasound, according to some embodiments.

In another aspect, at least one of the first or second fluids can be ruptured. The fluids can be ruptured, for example, to release a substance from the droplet. A fluidic portion is ruptured when it is divided into at least 5 or at least 10 separate droplets. In one embodiment, a droplet is ruptured in a manner that causes the droplet to release at least some of its contents. Typically, this happens on a very rapid time scale, often in an uncontrolled manner. In some cases, this may occur due to a change in phase (e.g., due to heating), due to rapid expansion of the fluid, or the like. Non-limiting examples of rupturing are included below.

In one embodiment of the invention, at least one of the first or second fluids is ruptured. In some cases, one of the first or second fluids is ruptured by the application of ultrasonic waves. In some embodiments, the ultrasonic frequency used to rupture the first or second fluid is at least about 20 kHz, at least about 500 kHz, at least about 1 MHz, or at least about 10 MHz, etc.

In other embodiments, the first or second fluids can be ruptured using heat. The heat may be applied from any suitable heat source. In some cases, the gas can be heated to a temperature of at least about 30 °C, at least about 40 °C, at least about 50 °C, at least about 100 °C, or at least about 200 °C, etc. As mentioned, any suitable method may be used to heat the gas. For example, the gas can be heated using localized heaters, a chemical reaction, ultrasonic waves, or the like.

Another aspect of the invention is directed to a method of administering a fluidic droplet and/or a multiple emulsion, such as those described herein, to a subject, such as a human subject. The subject may be, for instance, a human or non-human animal. Examples of subjects include, but are not limited to, a mammal such as a dog, a cat, a horse, a rabbit, a cow, a pig, a sheep, a goat, a rat (e.g., *Rattus Norvegicus*), a mouse (e.g., *Mus musculus*), a guinea pig, a hamster, a primate (e.g., a monkey, a chimpanzee, a baboon, an ape, a gorilla, etc.), or the like. The administration may be performed using any suitable technique, e.g., one that is medically accepted. The administration may be localized (i.e., to a particular region, physiological system, tissue, organ, or cell type) or systemic, depending on the condition to be treated. Examples of parenteral modalities that can be used with the invention include, but are not limited to, intravenous, intradermal, subcutaneous, intracavity, intramuscular, intraperitoneal, epidural, or intrathecal.

In one set of embodiments, the multiple emulsion is administered and ultrasound is applied to the subject. In some embodiments, ultrasound may be applied to the subject to acquire an ultrasound image of the subject. In some cases, the multiple emulsion acts as a contrast agent. For instance, if a portion of the multiple emulsion contains a gas such as air, ultrasound (e.g., diagnostic ultrasound, for instance, having a frequency of between about 2 and about 18 megahertz) may be applied to the subject. The multiple emulsion may also contain a hardened shell, as previously described, e.g., surrounding an inner "bubble" of gas. Without wishing to be bound by any theory, it is believed that such emulsions may improve ultrasound signal backscatter, e.g., for contrast-enhanced ultrasound, as it is believed that the gas within the multiple emulsion may allow the emulsion droplets to have a relatively high degree of echogenicity, which is the ability to reflect the ultrasound waves. Typically, the multiple emulsion is not ruptured when the ultrasound is applied.

In one set of embodiments the ultrasound applied to the subject may be performed to rupture the multiple emulsion. In some cases, as previously discussed, frequencies of at least 20 kHz, at least 500 kHz, at least 1 MHz, or at least 10 MHz may be used. For instance, the multiple emulsion may contain a drug or other therapeutic agent, which is released, systemically or locally, upon application of the ultrasound to the subject to which the multiple emulsion was administered. The rupturing of such multiple emulsions has been described, above. It should be noted, however, that other methods besides and/or in addition to ultrasound may be used to rupture the multiple emulsions administered to the subject. For instance, as previously described, heat may be applied to the multiple emulsion (e.g., heat may be applied to at least a portion of the multiple emulsion).

A variety of materials and methods, according to certain aspects of the invention, can be used to form systems (such as those described above) able to produce the multiple droplets described herein. In some embodiments, a microfluidic device is used to produce multiple droplets. The microfluidic devices can be fabricated using soft lithography. In addition, in some embodiments, the microfluidic device includes a T-junction, or a Y-junction, e.g., as is shown in the figures. In some cases, a multiple emulsion can be formed by directing a fluidic droplet within a first channel, containing an inner fluid, at a non-linear intersection of the first and a second channel. The second channel may contain a second fluid that is substantially immiscible with the fluidic droplet. Upon entering the second channel, the fluidic droplet may be encapsulated by the second fluid, thereby forming a multiple emulsion. A non-limiting example is shown in Fig. 1C, where a gas is contained within a water droplet, which in turn is directed into an oil phase. As used herein, a non-linear intersection of two (or more) channels is one in which the centerline axes of the two channels are not parallel. Thus, for instance, the non-linear intersection may be a "T" junction or a "Y" junction, etc. Non-limiting examples of such non-linear intersections may be seen in the figures.

In some cases, the various systems lend themselves to various methods. For example, various components can be formed from solid materials, in which the channels can be formed via micromachining, film deposition processes such as spin coating and chemical vapor deposition, laser fabrication, photolithographic techniques, etching methods including wet chemical or plasma processes, and the like. See, for example, *Scientific American*, 248:44-55, 1983 (Angell, et al). In one embodiment, at least a portion of the fluidic system is formed of silicon by etching features in a silicon chip. Technologies for precise and efficient fabrication of various fluidic systems and devices of the invention from silicon are known. In another embodiment, various components of the systems and devices of the invention can be formed of a polymer, for example, an elastomeric polymer such as polydimethylsiloxane ("PDMS"), polytetrafluoroethylene ("PTFE" or Teflon®), or the like.

Different components can be fabricated of different materials. For example, a base portion including a bottom wall and side walls can be fabricated from an opaque material such as silicon or PDMS, and a top portion can be fabricated from a transparent or at least partially transparent material, such as glass or a transparent polymer, for observation and/or control of the fluidic process. Components can be coated so as to expose a desired chemical functionality to fluids that contact interior channel walls, where the base supporting material does not have a precise, desired functionality. For example, components can be fabricated as illustrated, with interior channel walls coated with another material. Material used to fabricate various components of the systems and devices of the invention, e.g., materials used to coat interior walls of fluid channels, may desirably be selected from among those materials that will not adversely affect or be affected by fluid flowing through the fluidic system, e.g., material(s) that is chemically inert in the presence of fluids to be used within the device.

In one embodiment, various components of the invention are fabricated from polymeric and/or flexible and/or elastomeric materials, and can be conveniently formed of a hardenable fluid, facilitating fabrication via molding (e.g. replica molding, injection molding, cast molding, etc.). The hardenable fluid can be essentially any fluid that can be induced to solidify, or that spontaneously solidifies, into a solid capable of containing and/or transporting fluids contemplated for use in and with the fluidic network. In one embodiment, the hardenable fluid comprises a polymeric liquid or a liquid polymeric precursor (i.e. a "prepolymer"). Suitable polymeric liquids can include, for example, thermoplastic polymers, thermoset polymers, or mixture of such polymers heated above their melting point. As another example, a suitable polymeric liquid may include a solution of one or more polymers in a suitable solvent, which solution forms a solid polymeric material upon removal of the solvent, for example, by evaporation. Such polymeric materials, which can be solidified from, for example, a melt state or by solvent evaporation, are well known to those of ordinary skill in the art. A variety of polymeric materials, many of which are elastomeric, are suitable, and are also suitable for forming molds or mold masters, for embodiments where one or both of the mold masters is composed of an elastomeric material. A non-limiting list of examples of such polymers includes polymers of the general classes of silicone polymers, epoxy polymers, and acrylate polymers. Epoxy polymers are characterized by the presence of a three-membered cyclic ether group commonly referred to as an epoxy group, 1,2-epoxide, or oxirane. For example, diglycidyl ethers of bisphenol A can be used, in addition to compounds based on aromatic amine, triazine, and cycloaliphatic backbones. Another example includes the well-known Novolac polymers. Non-limiting examples of silicone elastomers suitable for use according to the invention include those formed from precursors including the chlorosilanes such as methylchlorosilanes, ethylchlorosilanes, phenylchlorosilanes, etc.

Silicone polymers are preferred in one set of embodiments, for example, the silicone elastomer polydimethylsiloxane. Non-limiting examples of PDMS polymers include those sold under the trademark Sylgard by Dow Chemical Co., Midland, MI, and particularly Sylgard 182, Sylgard 184, and Sylgard 186. Silicone polymers including PDMS have several beneficial properties simplifying fabrication of the microfluidic structures of the invention. For instance, such materials are inexpensive, readily available, and can be solidified from a prepolymeric liquid via curing with heat. For example, PDMSs are typically curable by exposure of the prepolymeric liquid to temperatures of about, for example, about 65 °C to about 75 °C for exposure times of, for example, about an hour. Also, silicone polymers, such as PDMS, can be elastomeric, and thus may be useful for forming very small features with relatively high aspect ratios, necessary in certain embodiments of the invention. Flexible (e.g., elastomeric) molds or masters can be advantageous in this regard.

One advantage of forming structures such as microfluidic structures of the invention from silicone polymers, such as PDMS, is the ability of such polymers to be oxidized, for example by exposure to an oxygen-containing plasma such as an air plasma, so that the oxidized structures contain, at their surface, chemical groups capable of cross-linking to other oxidized silicone polymer surfaces or to the oxidized surfaces of a variety of other polymeric and non-polymeric materials. Thus, components can be fabricated and then oxidized and essentially irreversibly sealed to other silicone polymer surfaces, or to the surfaces of other substrates reactive with the oxidized silicone polymer surfaces, without the need for separate adhesives or other sealing means. In most cases, sealing can be completed simply by contacting an oxidized silicone surface to another surface without the need to apply auxiliary pressure to form the seal. That is, the pre-oxidized silicone surface acts as a contact adhesive against suitable mating surfaces. Specifically, in addition to being irreversibly sealable to itself, oxidized silicone such as oxidized PDMS can also be sealed irreversibly to a range of oxidized materials other than itself including, for example, glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, glassy carbon, and epoxy polymers, which have been oxidized in a similar fashion to the PDMS surface (for example, via exposure to an oxygen-containing plasma). Oxidation and sealing methods useful in the context of the present invention, as well as overall molding techniques, are described in the art, for example, in an article entitled "Rapid Prototyping of Microfluidic Systems and Polydimethylsiloxane," *Anal. Chem.*, 70:474-480, 1998 (Duffy, et al.), incorporated herein by reference.

In some embodiments, certain microfluidic structures of the invention (or interior, fluid-contacting surfaces) may be formed from certain oxidized silicone polymers. Such surfaces may be more hydrophilic than the surface of an elastomeric polymer. Such hydrophilic channel surfaces can thus be more easily filled and wetted with aqueous solutions.

In one embodiment, a bottom wall of a microfluidic device of the invention is formed of a material different from one or more side walls or a top wall, or other components. For example, the interior surface of a bottom wall can comprise the surface of a silicon wafer or microchip, or other substrate. Other components can, as described above, be sealed to such alternative substrates. Where it is desired to seal a component comprising a silicone polymer (e.g. PDMS) to a substrate (bottom wall) of different material, the substrate may be selected from the group of materials to which oxidized silicone polymer is able to irreversibly seal (e.g., glass, silicon, silicon oxide, quartz, silicon nitride, polyethylene, polystyrene, epoxy polymers, and glassy carbon surfaces which have been oxidized). Alternatively, other sealing techniques can be used, as would be apparent to those of ordinary skill in the art, including, but not limited to, the use of separate adhesives, bonding, solvent bonding, ultrasonic welding, etc.

The following are each incorporated herein by reference: U.S. Patent Application Serial No. 11/246,911, filed October 7, 2005, entitled "Formation and Control of Fluidic Species," published as U.S. Patent Application Publication No. 2006/0163385 on July 27, 2006; U.S. Patent Application Serial No. 11/024,228, filed December 28, 2004, entitled "Method and Apparatus for Fluid Dispersion," published as U.S. Patent Application Publication No. 2005/0172476 on August 11, 2005; U.S. Patent Application Serial No. 11/360,845, filed February 23, 2006, entitled "Electronic Control of Fluidic Species," published as U.S. Patent Application Publication No. 2007/000342 on January 4, 2007; International Patent Application No. PCT/US2006/007772, filed March 3, 2006, entitled "Method and Apparatus for Forming Multiple Emulsions," published as WO 2006/096571 on September 14, 2006; U.S. Patent Application Serial No. 11/368,263, filed March 3, 2006, entitled "Systems and Methods of Forming Particles," published as U.S. Patent Application Publication No. 2007/0054119 on March 8, 2007; U.S. Provisional Patent Application Serial No. 60/920,574, filed March 28, 2007, entitled "Multiple Emulsions and Techniques for Formation"; International Patent Application No. PCT/US2006/001938, filed January 20, 2006, entitled "Systems and Methods for Forming Fluidic Droplets Encapsulated in Particles Such as Colloidal Particles," published as WO 2006/078841 on July 27, 2006; International Patent Application Serial No.: PCT/US2008/004097, filed March 28, 2008, entitled "Multiple Emulsions and Techniques for Formation," by Chu *et al.*; U.S. Provisional Patent Application Serial No. 60/997,994, filed October 5, 2007, entitled "Formation of Particles for Ultrasound Application, Drug Release, and Other Uses, and Microfluidic Methods of Preparation," by Stone, *et al.*; U.S. Provisional Patent Application Serial No. 60/997,996, filed October 5, 2007, entitled "Reactions Within Microfluidic Channels," by Stone, *et al.*; and an

International Patent Application filed on even date herewith, entitled "Reactions Within Microfluidic Channels," by Stone, *et al.*.

The following examples are intended to illustrate certain embodiments of the present invention, but do not exemplify the full scope of the invention.

EXAMPLE 1

This example illustrates a microfluidic technique for producing various multiple emulsions. Figures 1A-C illustrate the production of multiple emulsions in which a gas bubble is surrounded by a water bubble which is surrounded by oil. In this example, the gas was nitrogen, the water phase was deionized water with 2% SDS (sodium dodecyl sulfate), and the oil phase was light mineral oil. Gas broke into droplets at the intersection of two channels (see Fig. 1B), to form single emulsions. The outermost fluid was flowed through a channel that formed a T-junction with the original channel. The solution containing single emulsion droplets broke into drops at the T-junction (Fig. 1B), resulting in double emulsions. The resulting emulsions were substantially monodisperse (Fig 3F-G). The number of droplets contained inside the emulsions could be controlled by changing the flowrates of the emulsion components (Fig. 4A-D and Fig 5A-D).

EXAMPLE 2

This example illustrates another microfluidic technique for producing various multiple emulsions. Figures 2A-C illustrate the production of multiple emulsions in which a gas bubble is surrounded by a water bubble which is surrounded by oil. In this example, the gas was nitrogen, the water phase was deionized water with 2% SDS, and the oil phase was light mineral oil. Gas broke into droplets at the intersection of two channels (see Fig. 2B), to form single emulsions. The outermost fluid was flowed through a channel that formed a Y-junction with the original channel. The solution containing single emulsion droplets broke into drops at the Y-junction (Fig. 1B), resulting in double emulsions. The resulting emulsions were substantially monodisperse (Fig 3D-E).

EXAMPLE 3

In this example, micron-diameter water droplets were formed in a continuous oil phase, where each droplet encapsulated a discrete number of gas bubbles. The approach combined two different microfluidic geometries: flow-focusing and a T-junction (Fig. 6). In particular, monodisperse microbubbles were first generated in a continuous water phase using a flow-focusing geometry, after which the gas-water system was dispersed into a continuous oil phase either by a flow-focusing or a T-junction element so as to obtain water drops that contain individual gas bubbles. The generation of water-encapsulated microbubbles in both geometries was dependent on the flow rates of the two liquids, and it was less sensitive to the gas pressure in the double flow-focusing (DFF) geometry (Figs. 6C and 6D) than in the geometry with flow-focusing followed by a T-junction (FFT) (Figs. 6A and 6B). Moreover, the DFF was able to form a relatively thin water layer encapsulating individual microbubbles while the FFT, on the other hand, had the advantage of controlling of the *number* of bubbles per water droplet. This characteristic feature of FFT was illustrated using an aqueous photopolymerizable acrylamide solution. Monodisperse porous polyacrylamide particles were fabricated with relatively low elastic moduli compared with solid polyacrylamide particles. These ideas provide an avenue for systematically controlling gas-liquid microstructures of double-emulsion type and offer a new fabrication method for polymer-covered microbubbles and porous microparticles. In this example, the controlled formation of three-phase materials using microfluidic tools to obtain micron-dimension structuring was investigated. Such double or multiple emulsions are most commonly made using bulk processing techniques. Simple capsules are another example of a three-phase material (liquid, shell, liquid) made from liquid precursors. To achieve drop-by-drop control, individual microfluidic devices are usually considered. The detailed control of the microstructure enables novel routes for controlled release. The experiments described herein investigate extending the range of materials to include monodisperse gas-liquid multiple emulsions. A method for making gas-liquid-liquid emulsions, with control of the size and number of the encapsulated phase is introduced. Finally, the further step of polymerization of the compound drops to form monodisperse porous particles is reported.

Figs. 6A-6D include schematic diagrams and images of experimental setups used to generate gas-in-water-in-oil emulsions. Figs. 6A-6B include an illustration (Fig. 6A) and an experimental image (Fig. 6B) of water droplet encapsulating microbubbles in a flow-focusing then T-junction microfluidic device (FFT). The scale bar in Fig. 6B represents 200 microns in length. Figs. 6C-6D include an illustration (Fig. 6C) and an experimental image (Fig. 6D) of water droplet encapsulating microbubbles in a double flow-focusing microfluidic device (DFF). The scale bar in Fig. 6D represents 150 microns in length. All experiments illustrated in Figs. 6A-6D include 2% (w/w) SDS in the water phase.

First, the device with flow focusing followed by a T-junction, or FFT, was produced, which allows the controlled formation of multiple micron-size bubbles per droplet with the coefficient of variation less than 0.01. In particular, it is shown in Fig. 7, that the number of encapsulated bubbles, all of roughly the same size, in each water droplet, was controlled by the flow rate ratio of water to oil (Q_w/Q_o) at constant gas pressure. It has been found that conditions can be identified that reproducibly give discrete numbers of gas bubbles per droplet. For example, systems have been made that give one (Fig. 7A), two (Fig. 7B), three (Fig. 7C), four (Fig. 7D) or even more bubbles per droplet. In Figs. 7A-7D, the pressure of the gas phase was constant at 18 psi (1 psi = 6.89475 kilopascals). The gas bubbles did not coalesce. In addition, it was observed that the gas bubbles tended to be organized near the front of the drop (the small dark arrows indicate the flow direction in each channel), which was consistent with the flow inside the drop and the fact that the bubbles were sufficiently large that they could not recirculate. Furthermore, by adjusting the gas pressure, as shown in Fig. 7E regimes where the number of encapsulated bubbles were either independent of the gas pressure ($Q_w/Q_o = 0.3\text{-}0.6$) or dependent on the gas pressure ($Q_w/Q_o = 0.6\text{-}0.9$; $0.25\text{-}0.3$) were observed. Fig. 7E is a plot of the number of encapsulated microbubbles (N) as a function of the flow rate ratio of water to oil (Q_w/Q_o) at two different gas pressures (▲ (closed triangles): 18 psi. Δ (open triangles): 20 psi). If the flow rate ratio of water to oil was lower than 0.15 or higher than 1, operation was beyond the regime where gas-in-water-in-oil emulsions could form steadily and gave either a string of gas bubbles in a continuous water phase (Fig. 7F) or water droplets with no bubbles at all. In Fig. 7F, the train of gas bubbles in a continuous stream of the water phase was formed using Q_w/Q_o = 1.6. The dark spheres are gas bubbles and the continuous phase is mineral oil. The small dark arrows indicate the flow direction in each channel. The scale bar for Fig. 7F represents 200 microns in length.

The effect of the flow rates and gas pressure on the formation of the 'single bubble per drop' regime was then investigated in both FFT and DFF devices. In Figures 8A and 8C, the drop and bubble diameter (d) relative to the orifice width (D) was plotted at different gas pressures as a function of the flow rate ratio of water to oil (Q_w/Q_o) in FFT and DFF, respectively. In Fig. 8A, the following symbols are used: solid square = droplet at 18 psi; solid circle = bubble at 18 psi; open square = droplet at 20 psi; and open circle = bubble at 20 psi. In Fig. 8C, the following symbols are used: open square = droplet at 13 psi; open circle = bubble at 13 psi; solid square = droplet at 18 psi; solid circle = bubble at 18 psi; plus in square = droplet at 19 psi; plus in circle = bubble at 19 psi; x in square = droplet at 20 psi; and x in circle = bubble at 20 psi. It was observed that, once the gas-in-water-in-oil emulsions were formed in both devices, the sizes of the encapsulated bubbles were strongly dependent on the relative flow rate (Q_w/Q_o), but the sizes of mother droplets did not depend significantly on Q_w/Q_o. This feature allows control of the thickness of the water layer. It was also observed that the gas pressure played a minor role in the DFF device, but it significantly affected the droplet size in the FFT device, which is consistent with the reported role of the gas pressure in the break-up mechanism in a typical T-junction geometry. By plotting the scaled bubble diameter (d_{bubble}) relative to the drop diameter (d_{drop}) as a function of Q_w/Q_o in both FFT and DFT devices (Fig. 8B and 8D), a dependence of the thickness of the water layer on the relative flow rate was observed in both devices.

In Fig. 8B, the following symbols are used: solid square = 18 psi and open square = 20 psi. In Fig. 8D, the following symbols are used: open square = 13 psi; solid square = 18 psi; plus in square = 19 psi; and x in square = 20 psi. For the DFF device, when $Q_w/Q_o = 0.35$ 90 micrometer diameter gas bubbles surrounded by 17 micrometer thick water layers were obtained. Upon changing the relative flow rate to unity, water layers with thickness as small as 5 micrometers and encapsulated bubbles with diameter around 20 micrometers were measured, as shown in Fig. 8E. This control of the thickness of an aqueous layer provided a microfluidic route for the fabrication of microbubbles covered with thin layers of polymer. In Fig. 8E, the image of the formation of thin water layers (~ 5 micrometer) that encapsulated microbubble emulsions was taken with $Q_w/Q_o = 1$ and a pressure 14 psi in DFF. The dark spheres are gas bubbles and the continuous phase is mineral oil. The scale bar in Fig. 8E represents a length of 150 micrometers.

Flow conditions in the FFT where a large number of gas bubbles are produced in each water droplet have also been identified. Porous particles were fabricated by first making droplets containing many gas bubbles and then polymerizing the aqueous phase.

Figs. 9A and 9B, respectively, show the sequential generation of photopolymerizable acrylamide aqueous droplets with encapsulated microbubbles ($Q_w/Q_o = 1$) and the collection of these droplets in the reservoir before UV irradiation. Fig. 9A includes an image of the sequential formation of multi-bubbles per drop in a flow-focusing followed by a T-junction device (FFT) with $Q_w/Q_o = 1$ and $P = 18$ psi. The scale bar in Fig. 9A represents a length of 200 microns. Fig. 9B includes an inverted image of collected drops with encapsulated microbubbles. The scale bar in Fig. 9B represents a length of 200 microns. Fig. 9C shows a magnified view of one acrylamide aqueous droplet after UV irradiation and many trapped bubbles are evident. The scale bar in Fig. 9C represents a length of 50 microns. The objects are effectively microspheres of a closed-cell foam. Once the bubbles were trapped inside water droplets or the polymerized polyacrylamide particles, they were stable for as long as 60 minutes before significant dissolution was evident.

The effective elastic modulus of the dry photopolymerized polyacrylamide particles with and without entrapped microbubbles was investigated using atomic force microscope (AFM) (Fig. 9D). Fig. 9D is a plot of the force indentation curves of dry polyacrylamide particles when photopolymerized with (open square, lower curve) and without bubbles (open circle, upper curve). The black solid lines represent the fit by the tip model (equation 1, as described later) to the indentation data on the polyacrylamide particles when photopolymerized with bubbles (lower curve, open square, $E = 3.6 \pm 1.2 \times 10^7$ Pa) and without bubbles (upper curve, open circles, $E_s = 8.6 \pm 1.7 \times 10^8$ Pa).

The porous polyacrylamide particles showed relatively low elastic moduli ($E = 3.6 \pm 1.2 \times 10^7$ Pa) compared to the particles without bubbles ($E_s = 8.6 \pm 1.7 \times 10^8$ Pa). Note that $E/E_s = 0.04$. An estimate of the gas fraction obtained by approximating the number of gas bubbles in the porous particles gives an effective density of $0.27\rho_s$ (rho-s), where ρ_s (rho-s) is the density of the polymerized polyacrylamide. These results were consistent with macroscopic measurements of the elasticity of other closed-cell foams. Thus, the ability to tune the number of gas bubbles per droplet offers an approach to manipulate the elasticity of individual microparticles by changing the internal porosity.

Fabrication of Microfluidic Devices and Experimental Setup: Microfluidic chips were fabricated in PDMS using standard soft photolithography techniques. The water and oil were loaded in two syringes (Hamilton) respectively and connected to syringe pumps (Kd Scientific, KDS101). Pressure was applied to the needle independently controlled by a regulator (Bellofram, St. Louis, MO) with a precision of 0.1 psi. Polyethylene (PE 20) tubes were connected from the syringe needle to the inlet hole of the channel of the device. Before use, the microfluidic chips were treated with octadecyltrichlorosilane (OTS) to make the glass surface hydrophobic.

The illustrations of the double flow-focusing (DFF) and flow-focusing followed by a T-junction (FFT) microfluidic devices are shown in Fig. 6. The height of the channels was everywhere equal 38 micrometers as measured with surface profilometer. The widths of the gas and water channels were 100 micrometers; the width of the central channel where gas bubbles were dispersed in the water phase was 60 micrometers; the width of the oil channel was 150 micrometers (DFF) or 200 micrometers (FFT); and the widths of the orifices for all geometries were either 20 or 30 micrometers. For the flow-rate dependent measurements, pure nitrogen gas bubbles were dispersed into deionized water with sodium dodecyl sulfate (SDS) (2 wt%, Aldrich) after which the water droplets with bubble(s) were dispersed into the mineral oil (Aldrich).

For the photopolymerization reactions, acrylamide (36 wt%, Aldrich), *N,N*-methylenebisacrylamide (1.5 wt%, Aldrich) and 2,2-diethoxyacetophenone (0.5 wt%, Aldrich) were dissolved in the water phase (deionized water) and 2,2-diethoxyacetophenone (5 wt%) was dissolved in the oil phase (PDMS fluid 200 and 749, Dow Corning). The acrylamide droplets were polymerized by activating the photoinitiator using a 100 W mercury lamp coupled into a 10x microscope lens (NA = 0.25) on an inverted fluorescence microscope (Leica, Bannockburn, IL).

Microbubble emulsions were directly observed using a high-speed video camera (Phantom V 9, 1400 frames per second) mounted on the microscope. The size distributions of the droplets and encapsulated bubbles were analyzed using an image analysis program written in-house with Matlab software.

Atomic Force Microscope Measurement: Elastic properties of the dry polyacrylamide particles were characterized by indentation measurements on a MFP-3D Coax atomic force microscope (AFM) coupled with an invert microscope (Asylum Research, Santa Barbara, CA). A silicon nitride probe (MikroMash, OR) with a force constant of ~ 0.15 N m^{-1} was applied in the force mode. After the measurement, the collected force curves were converted into force versus indentation graphs using software provided by Asylum Research. The elastic moduli of the particles were determined by assuming a conical tip shape, which produces a load-indentation dependence

$$F = \frac{2E \tan \alpha}{\pi\left(1 - \upsilon^2\right)} \delta^2 \tag{1}$$

where F is the loading force (N), δ (delta) is the indentation (m), E is Young's modulus (Pa), υ is the Poisson's ratio (0.5), and α (alpha) is the tip semivertical angle (35°).

While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as "comprising," "including," "carrying," "having," "containing," "involving," "holding," "composed of," and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of" shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

What is claimed is:

CLAIMS

1. A method comprising:

   providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third fluid that is a liquid, at least one of the first fluid and the second fluids being a gas; and

   altering the volume of the gas by applying ultrasound to at least a portion of the gas.

2. A method as in claim 1, wherein the first fluid is a gas and the second fluid is a liquid.

3. A method as in claim 1, wherein the first fluid is a liquid and the second fluid is a gas.

4. A method as in claim 3, wherein the second fluid and the third fluid are substantially immiscible.

5. A method as in claim 1, wherein the ultrasound has an average frequency of at least about 20 kHz.

6. A method as in claim 1, wherein the ultrasound has an average frequency of at least about 500 kHz.

7. A method as in claim 1, wherein the ultrasound has an average frequency of at least about 1 MHz.

8. A method as in claim 1, wherein the ultrasound has an average frequency of at least about 10 MHz.

9. A method as in claim 1, wherein at least one of the first fluid and the second fluid comprises air.

10. A method as in claim 1, wherein at least one of the first fluid and the second fluid comprises nitrogen (N_2).

11. A method as in claim 1, wherein the first fluid comprises a polymer.

12. A method as in claim 1, wherein the first fluid comprises a gel.

13. A method as in claim 1, wherein the first fluid comprises a hydrogel.

14. A method as in claim 1, wherein the first fluid comprises polyacrylamide.

15. A method as in claim 1, wherein the third fluid comprises water.

16. A method as in claim 1, wherein the first fluid contains a biochemical species.

17. A method as in claim 1, wherein the second fluid contains a biochemical species.

18. A method as in claim 1, wherein the third fluid is contained within a microfluidic device.

19. A method as in claim 18, wherein the microfluidic device is fabricated using soft lithography.

20. A method as in claim 1, wherein altering comprises causing the volume of gas to oscillate.

21. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 1 Hz.

22. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 10 Hz.

23. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 100 Hz.

24. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 1 kHz.

25. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 10 kHz.

26. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 100 kHz.

27. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 1 MHz.

28. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 10 MHz.

29. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 1 GHz.

30. A method as in claim 20, wherein the volume of the gas oscillates at a frequency of at least about 10 GHz.

31. A method comprising:

providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third fluid that is a liquid, wherein at least one of the first fluid and the second fluid is a gas; and

altering the volume of the gas by at least about 5 percent.

32. A method as in claim 31, wherein altering the volume of the gas comprises applying ultrasound to at least a portion of the gas.

33. A method as in claim 32, wherein the ultrasound has an average frequency of at least about 20 kHz.

34. A method as in claim 32, wherein the ultrasound has an average frequency of at least about 1 MHz.

35. A method as in claim 31, wherein altering the volume of the gas comprises rupturing the gas.

36. The method of claim 31, wherein altering the volume of the gas comprises dividing the gas into at least five separate droplets.

37. A method as in claim 31, comprising altering the volume of the gas by at least about 10 percent.

38. A method as in claim 31, comprising altering the volume of the gas by at least about 50 percent.

39. A method as in claim 31, comprising altering the volume of the gas by at least about 100 percent.

40. A method as in claim 31, comprising altering the volume of the gas by at least about 200 percent.

41. A method as in claim 31, comprising altering the volume of the gas by at least about 500 percent.

42. A method as in claim 31, wherein altering the volume of the gas comprises heating the gas.

43. A method as in claim 42, wherein the gas is heated to a temperature of at least about 40 °C.

44. A method as in claim 42, wherein the gas is heated to a temperature of at least about 50 °C.

45. A method as in claim 42, wherein the gas is heated to a temperature of at least about 100 °C.

46. A method as in claim 42, wherein the gas is heated to a temperature of at least about 200 °C.

47. A method as in claim 42, wherein the gas is heated to a temperature of at least about 500 °C.

48. A method as in claim 42, wherein heating the gas comprises causinga chemical reaction to occur that alters the volume of the gas.

49. A method as in claim 42, wherein heating the gas comprises expoisng at least a portion of the gas to ultrasound.

50. A method as in claim 31, wherein the first fluid is a gas and the second fluid is a liquid.

51. A method as in claim 31, wherein the first fluid is a liquid and the second fluid is a gas.

52. A method, comprising:

providing a first fluid surrounded by a second fluid, the second fluid being surrounded by a third fluid that is a liquid; and

causing at least one of the first fluid and the second fluid to rupture.

53. A method as in claim 52, wherein one of the first fluid and the second fluid is a gas.

54. A method as in claim 52, wherein the first fluid is a gas and the second fluid is a liquid.

55. A method as in claim 52, wherein the first fluid is a liquid and the second fluid is a gas.

56. A method as in claim 52, wherein the rupturing is caused by applying ultrasound to at least one of the first fluid and the second fluid.

57. A method as in claim 56, wherein the ultrasound has an average frequency of at least about 20 kHz.

58. A method as in claim 52, wherein the rupturing is caused by applying heat to at least one of the first fluid and the second fluid.

59. A method as in claim 52, wherein the rupturing is caused by a chemical reaction.

60. A method, comprising:

administering, to a subject, a multiple emulsion having at least two nested fluids; and

applying ultrasound to the subject.

61. The method of claim 60, further comprising causing at least a portion of the multiple emulsion to rupture by applying ultrasound to the subject.

62. The method of claim 60, further comprising acquiring an ultrasound image of the subject.

63. The method of claim 60, wherein the subject is human.

64. A method, comprising:

administering, to a subject, a multiple emulsion having at least two nested fluids; and

thereafter, rupturing at least one of the fluids contained within the multiple emulsion.

65. The method of claim 64, comprising heating the multiple emulsion to cause the rupturing to occur.

66. The method of claim 64, comprising applying ultrasound to at least a portion of the multiple emulsion to cause the rupturing to occur.

67. The method of claim 64, wherein the subject is human.

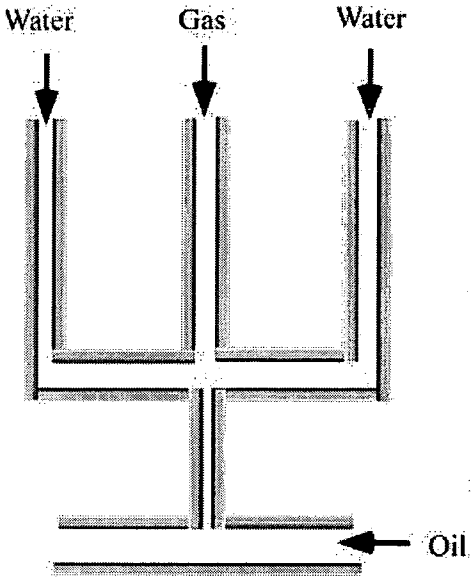


Fig. 1A

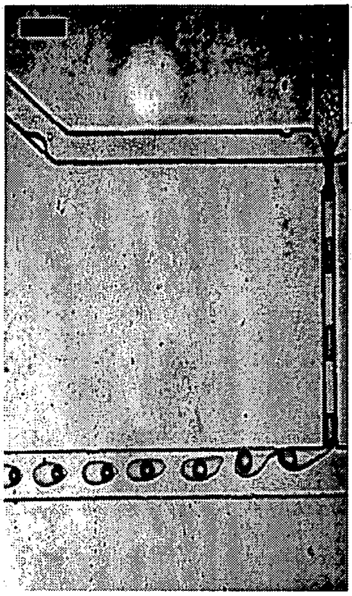


Fig. 1B

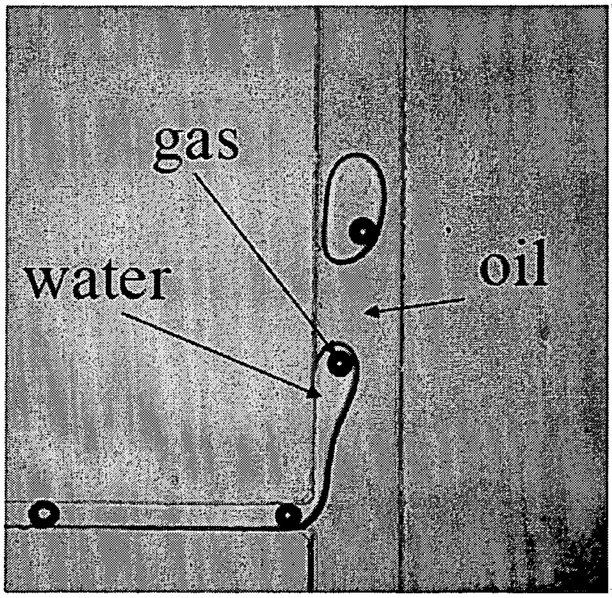


Fig. 1C

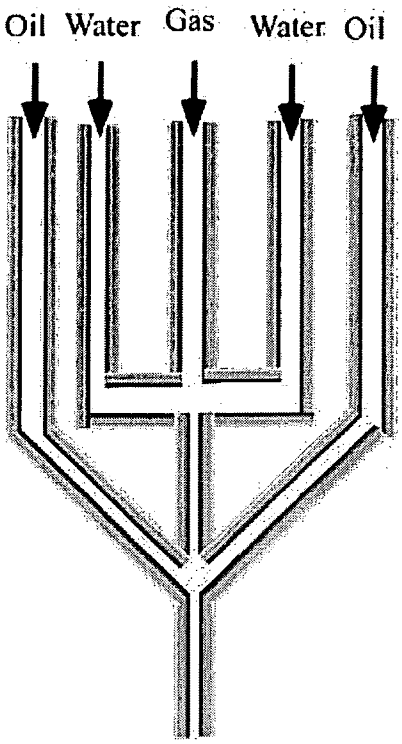


Fig. 2A

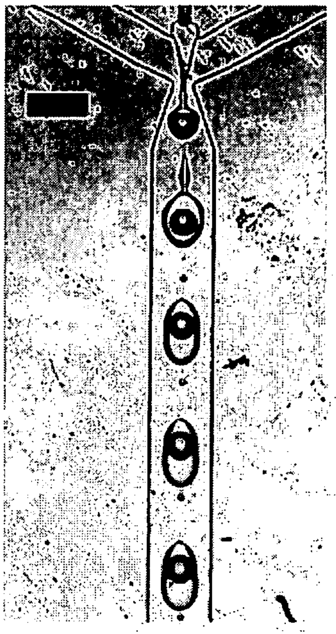


Fig. 2B

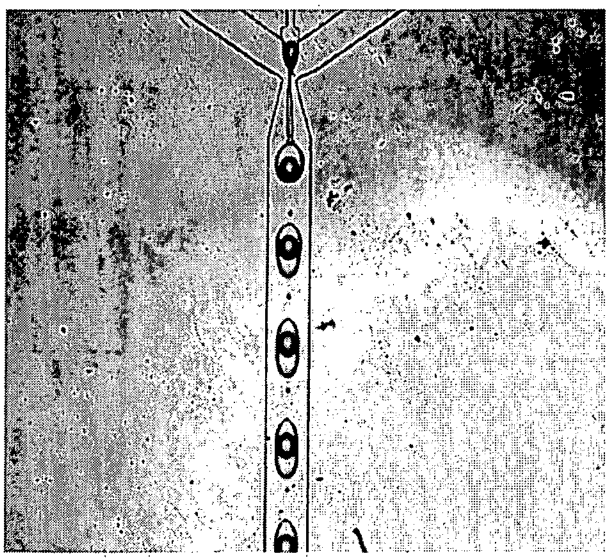
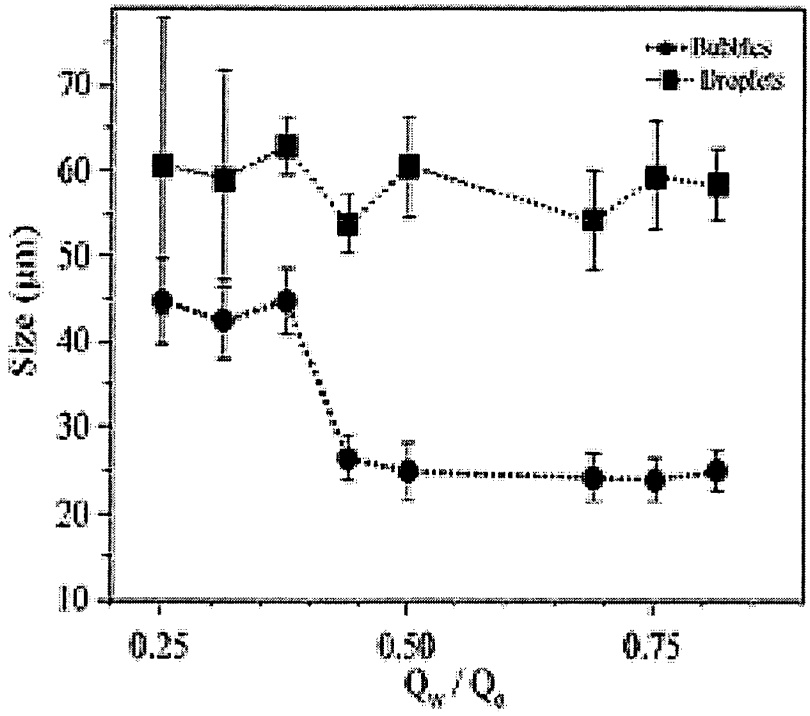


Fig. 2C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50
40
30
20
10
Size (μm)
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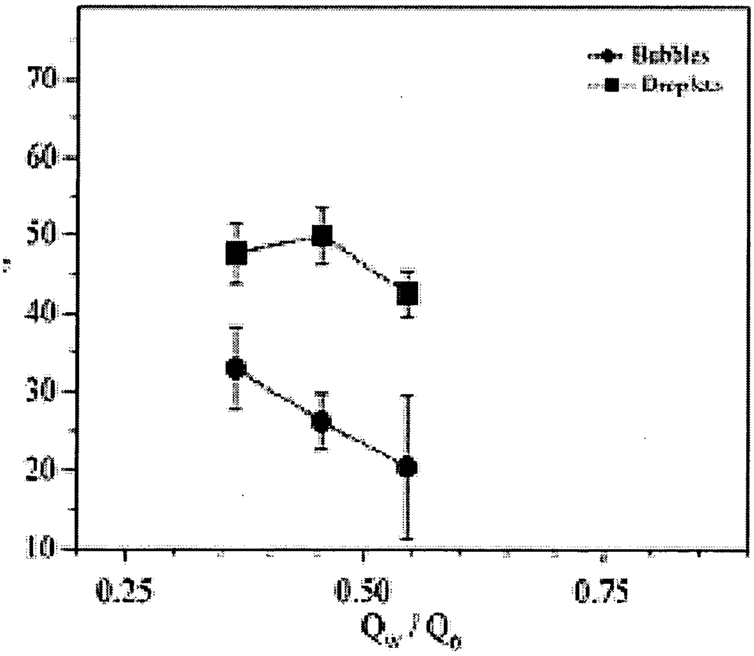
0.50
0.75
Q_w / Q_o

Fig. 3A


Bubbles
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Q_w / Q_o

Fig. 3B

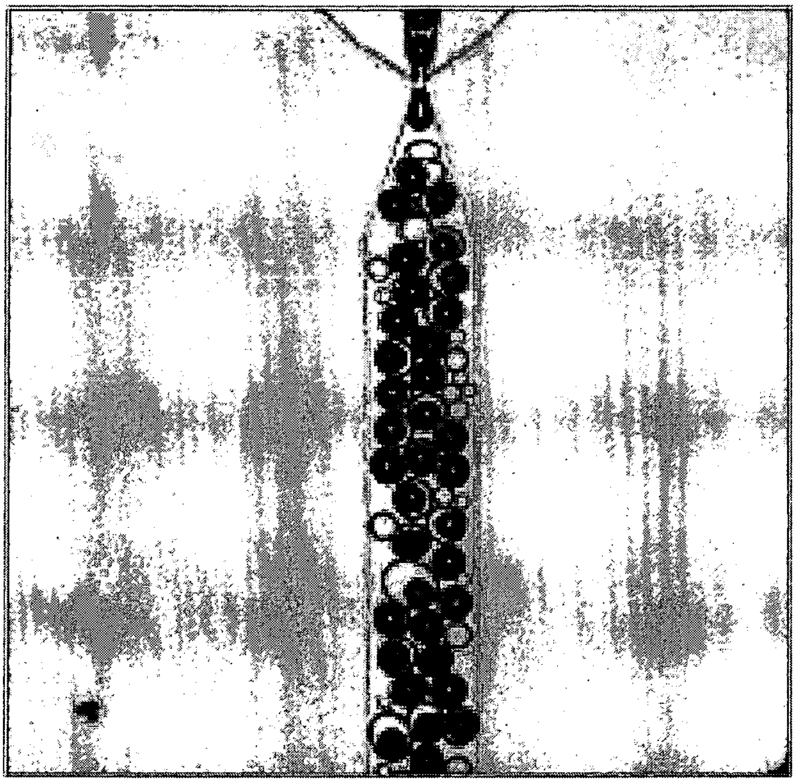
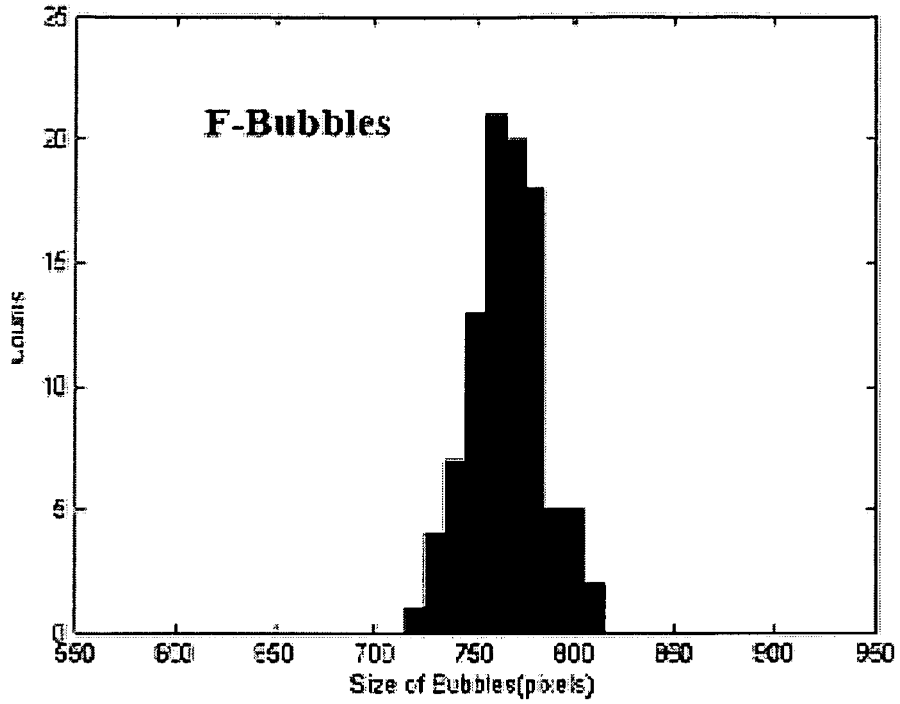


Fig. 3C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650
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750
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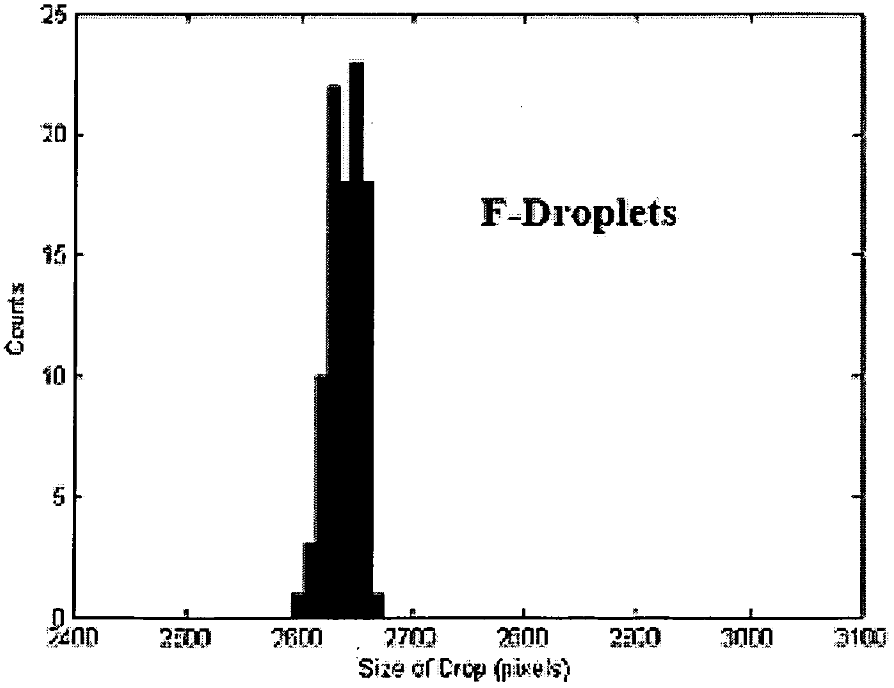
850
900
950
Size of Bubbles(pixels)

Fig. 3D


F-Droplets
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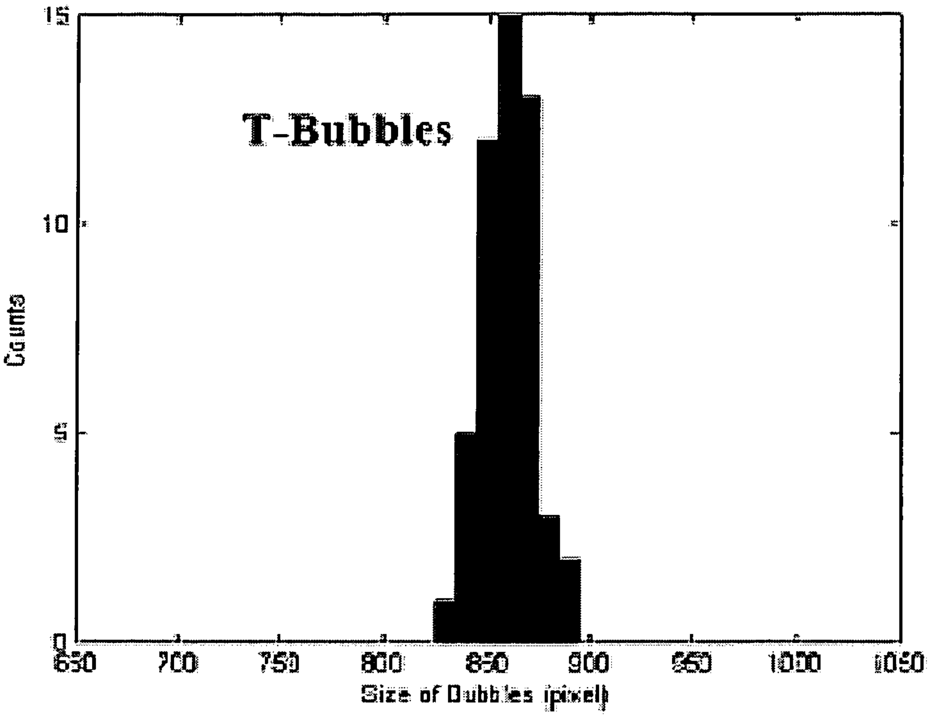
2600
2700
2800
2900
3000
3100
Size of Drop (pixels)

Fig. 3E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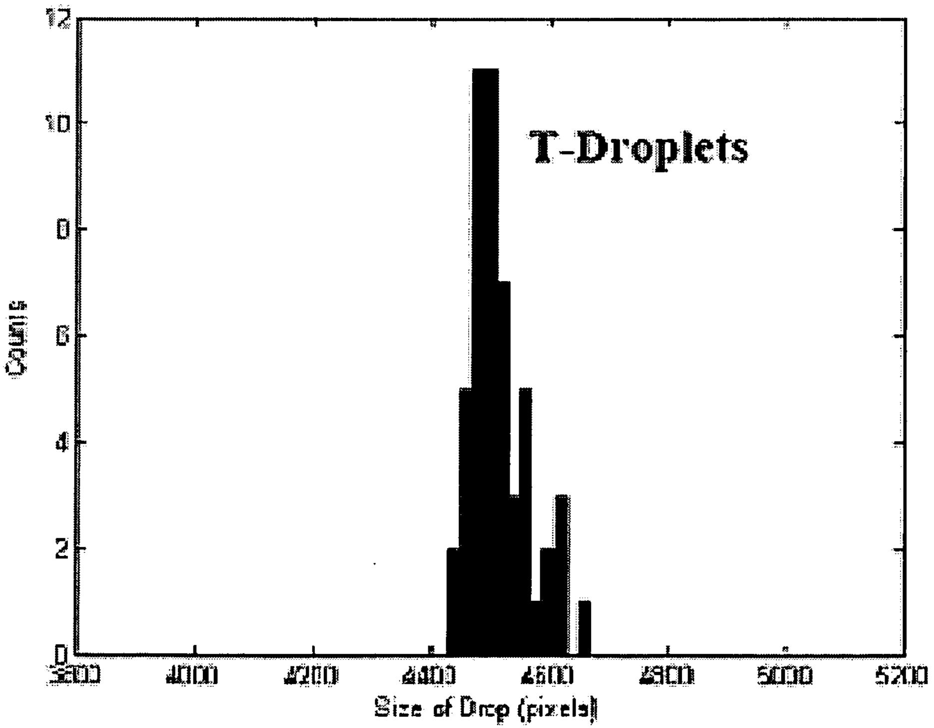
850
900
950
1000
1050
Size of Bubbles (pixel)

Fig. 3F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4000
4200
4400
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5200
Size of Drop (pixels)

Fig. 3G

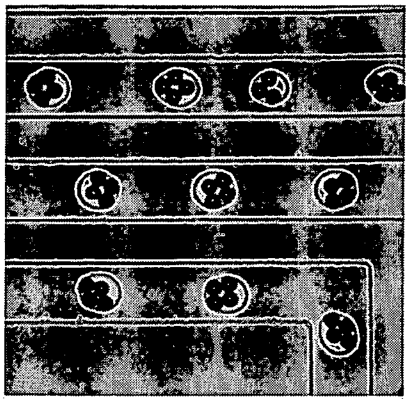

Fig. 4A

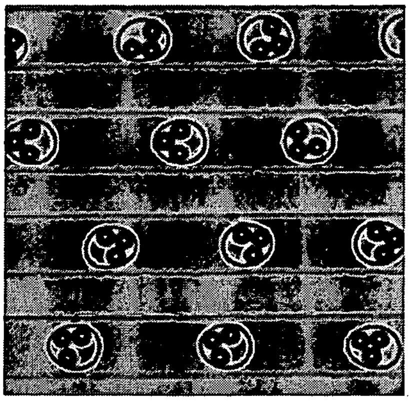

Fig. 4B

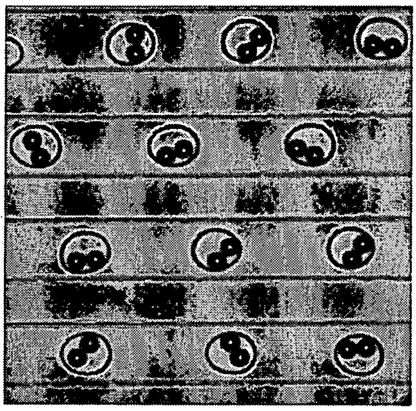

Fig. 4C

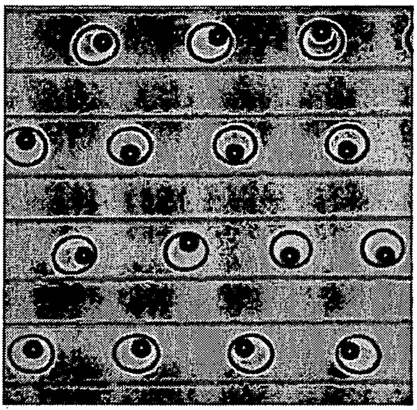

Fig. 4D

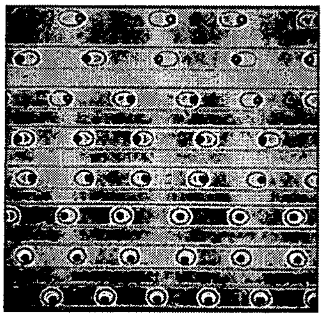

Fig. 4E

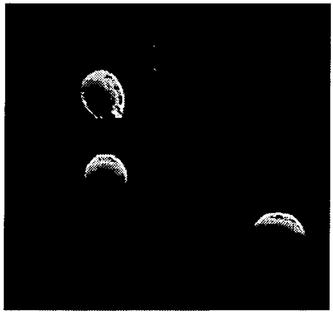

Fig. 4F

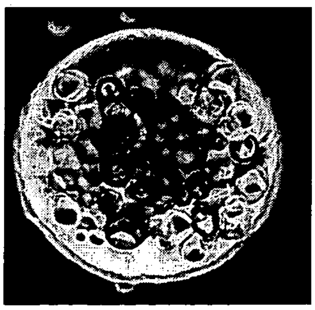

Fig. 4G

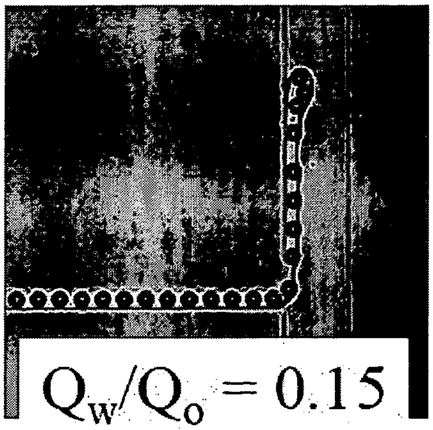

Fig. 5A

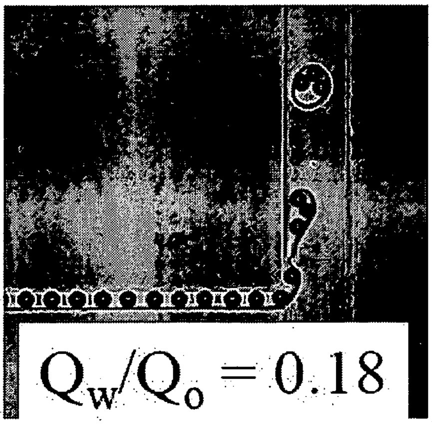

Fig. 5B

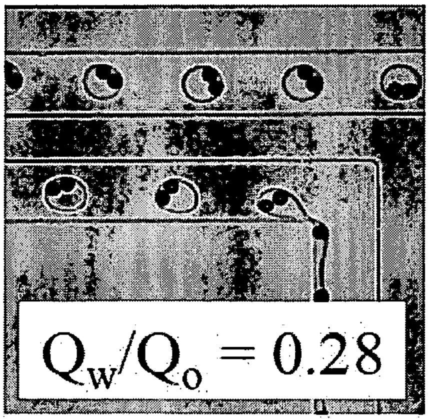

Fig. 5C

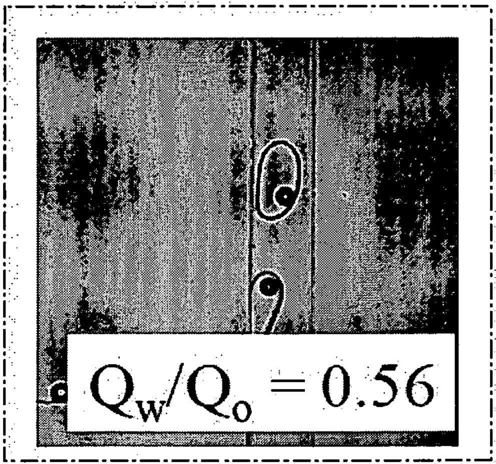

Fig. 5D

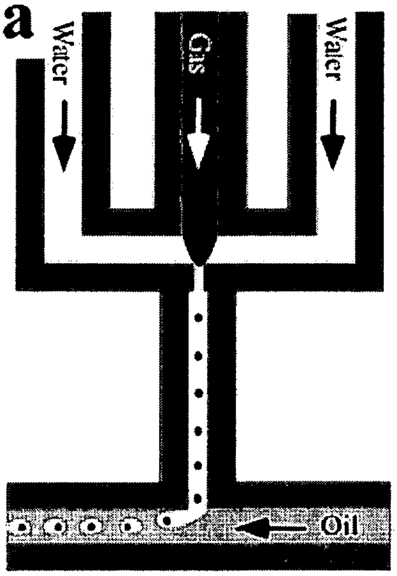

Fig. 6A

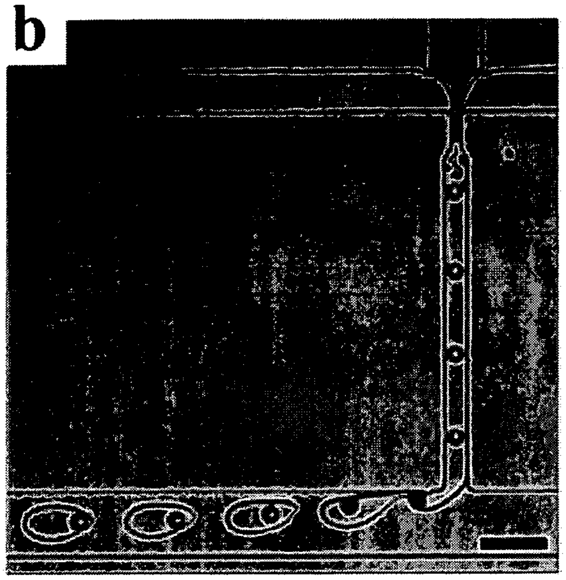

Fig. 6B

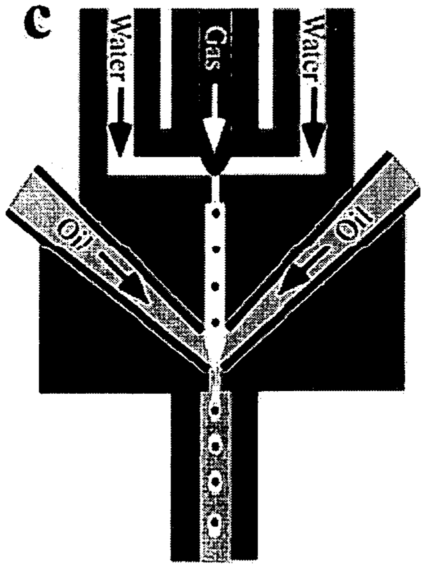

Fig. 6C

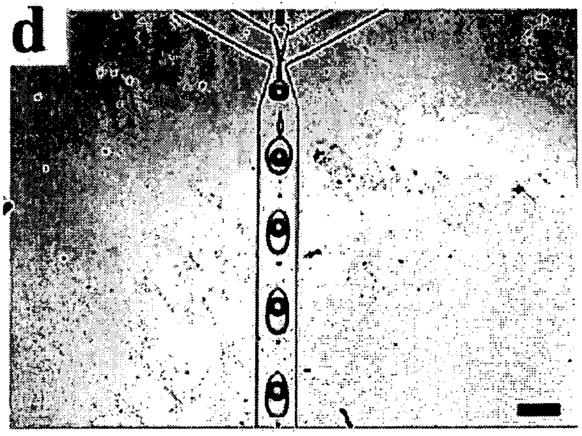

Fig. 6D

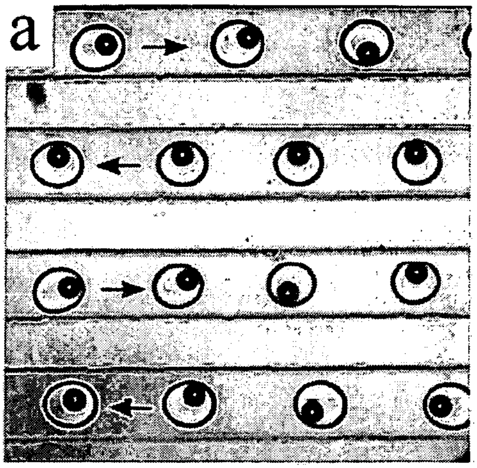

Fig. 7A

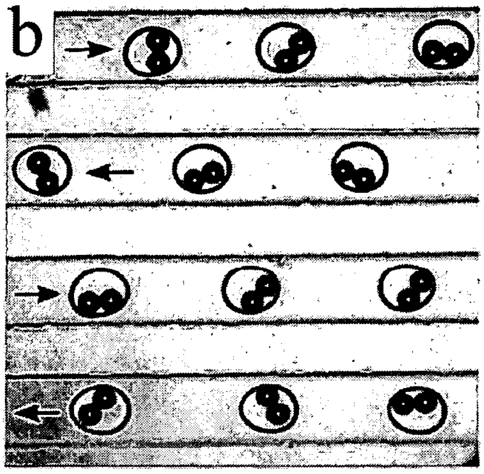

Fig. 7B

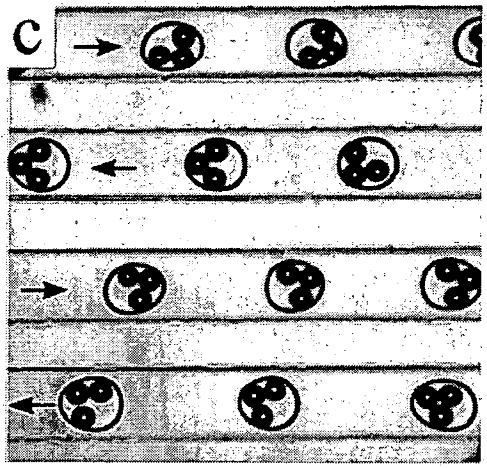

Fig. 7C

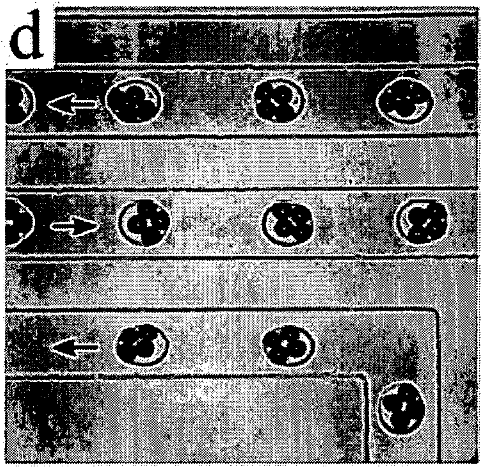

Fig. 7D

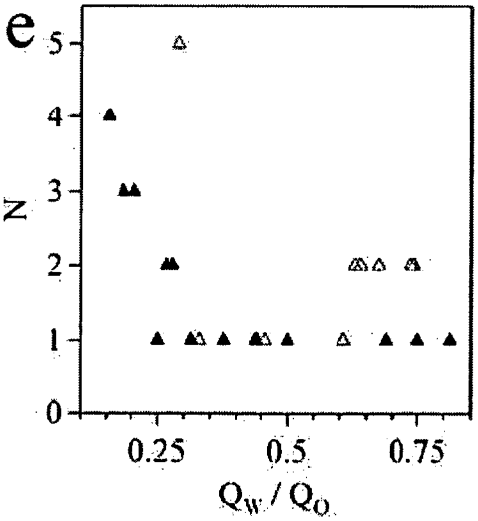

Fig. 7E

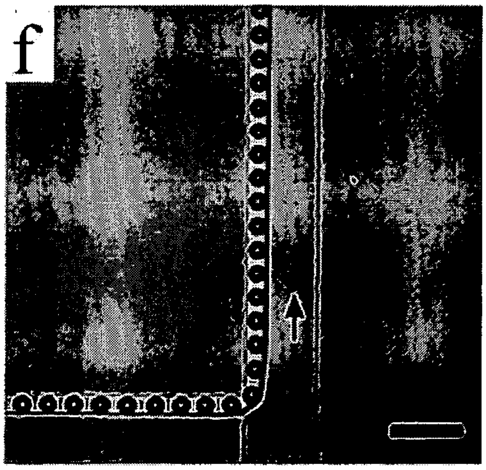

Fig. 7F

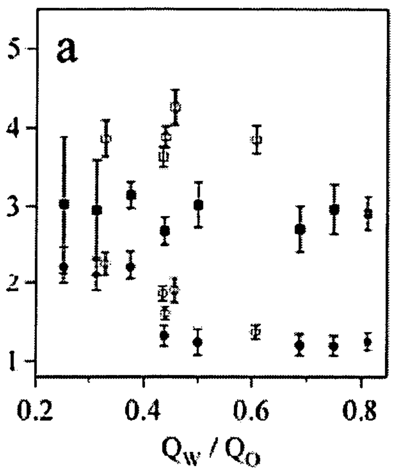

Fig. 8A

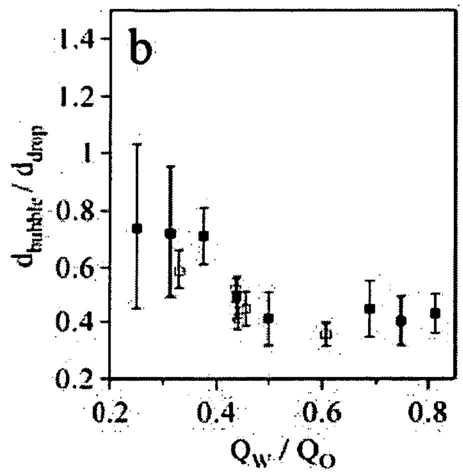

Fig. 8B

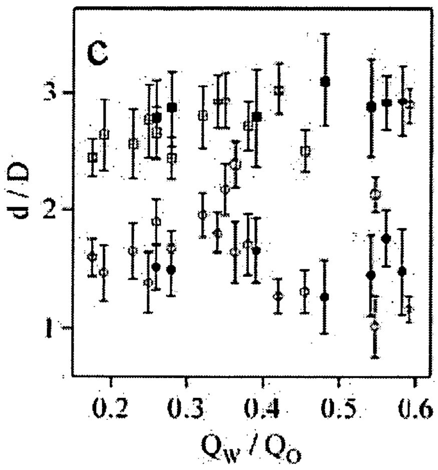

Fig. 8C

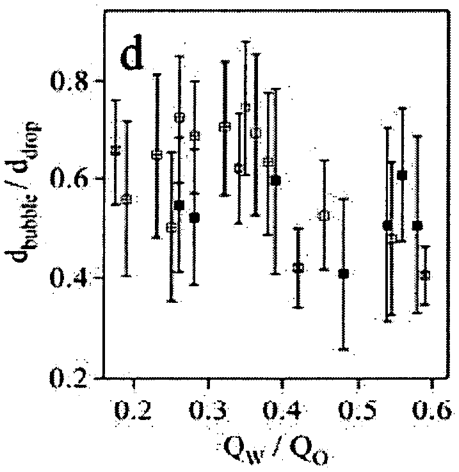

Fig. 8D

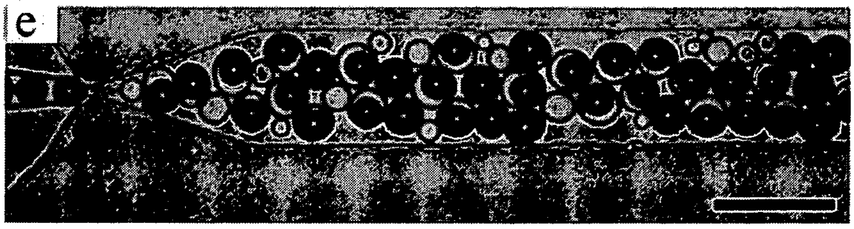

Fig. 8E

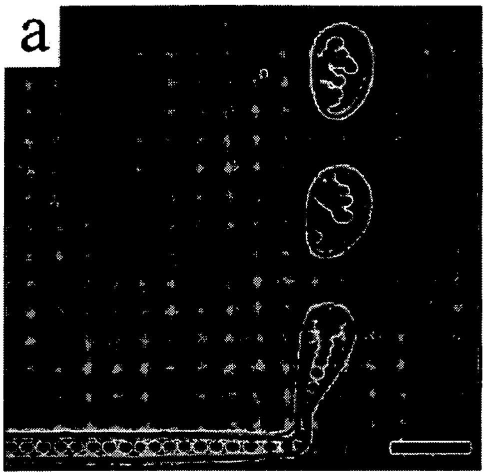

Fig. 9A

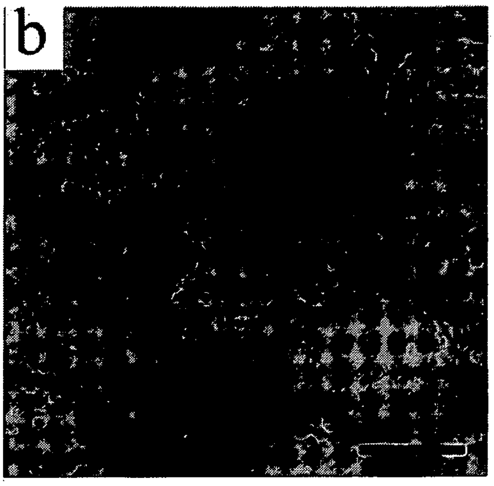

Fig. 9B

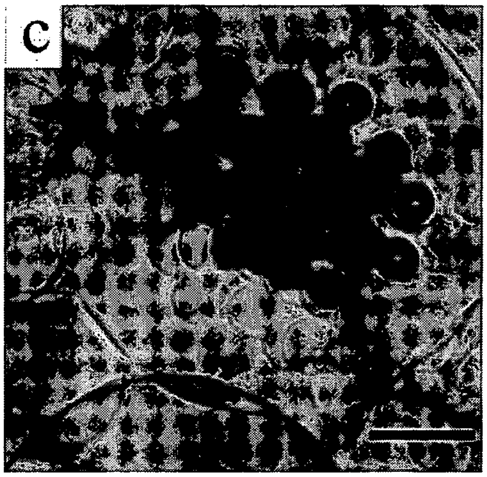

Fig. 9C

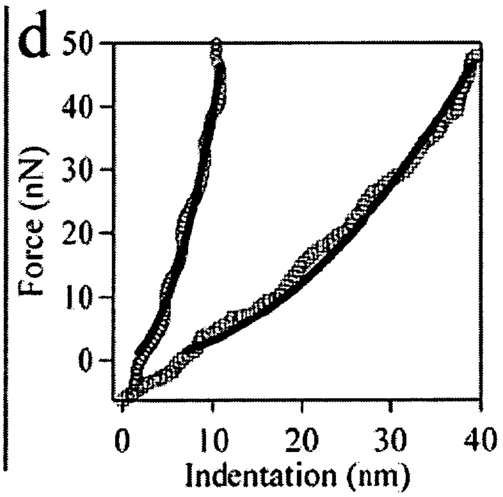

Fig. 9D