Published in final edited form as:

J Colloid Interface Sci. 2010 April 15; 344(2): 521–527. doi:10.1016/j.jcis.2010.01.042.

Polymer-Lipid Microbubbles for Biosensing and the Formation of Porous Structures

Kanaka Hettiarachchi^{a,*} and Abraham P. Lee^{a,b}

^aDepartment of Biomedical Engineering, University of California at Irvine, Irvine, CA 92697, USA. khettiar@uci.edu

^bDepartment of Mechanical and Aerospace Engineering, University of California at Irvine, Irvine, CA 92697, USA. Tel: +1-949-824-1036; aplee@uci.edu

Abstract

Polymer-lipid microbubbles (PLBs) are generated by microfluidic flow-focusing devices to form a new class of long-lasting hybrid particles. The specific PLB construct developed is an elastic gas-filled microsphere with a polydimethylsiloxane (PDMS) shell containing phospholipids conjugated to functionalized polyethyleneglycol (PEG). Digital “droplet-based” microfluidics technology enables control of particle composition, size, and polydispersity ($\sigma < 10\%$). Use of PDMS as a shell component improves the functionality and stability (lifetime > 6 months) of the hybrid particles due to the thermally maneuverable solidification process. With a gas core, they serve as a template material for creating three-dimensional porous structures and surfaces, requiring no cumbersome post-processing removal steps. By adding biotinylated PEG-lipid derivatives that offer targeting capabilities, we demonstrate the immobilization of fluorescent IgG antibodies on stationary PDMS-lipid microbubbles through biotinavidin interactions and on-chip trapping for immunoassays. A PDMS-lipid composition offers several advantages such as biocompatibility and biodegradability for future in-vivo use as porous engineered scaffolds, packing materials, or delivery (i.e. therapeutic) agents with cell targeting capability.

Keywords

Hybrid materials; Microbubble; Microfluidics; Polydimethylsiloxane; Polymer Microspheres

1. Introduction

Polymeric microparticle constructs are important engineered systems with a multitude of applications [1]. They can exhibit distinct differences regarding the makeup of the outer shell and inner core. Microbubbles represent an emerging class of particles with uses in diagnostics and therapeutics [2,3]. In general, novel particles for biosensing [4] and production of porous structures [5] are still however in the exploratory stages of development. Compressible

© 2009 Elsevier Inc. All rights reserved.

*Corresponding author: Kanaka Hettiarachchi, Department of Biomedical Engineering, University of California at Irvine, 3120 Natural Sciences II, Irvine, CA 92697-2715, USA, Phone: (650)-291-5299, Fax: (949) 824-1727, khettiar@uci.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version.

microbubbles can achieve very high surface area to volume ratios for a given size by dense packing arrangements [6]. The possibility therefore exists for improving analyte capture and detection by functionalizing individual particles. Utilizing fluorescent IgG antibodies as binding agents leads to high specificity since they recognize and selectively bind to relatively large bodies such as cells [7]. The lifetime of typical microbubble lipospheres however has been limited to a few hours upon production due to gas exchange and dissolution [8]. Armored bubbles that are encapsulated by a close-packed monolayer of solid particles driven by chemically assembly have improved stability by suppressing gas dissolution [9].

Microfluidic lab-on-a-chip devices have a multitude of uses, from on-chip separation [10] and high-throughput screening [11] to monodisperse emulsion generation [12,13] and chemical and material synthesis [14]. Although polydimethylsiloxane (PDMS) is widely used for microfluidic applications [15], little has been reported on the preparation and use of porous PDMS matrices [16] and early work on creating porous spherical particles with an open structure (where PDMS is the dispersed phase) was based on a bulk emulsion crosslinking procedure [17]. Porous filters based on emulsion polymerization have been fabricated inside microchannels [18]. Microfluidic devices have also been used as biosensors for detecting various pathogens [19–21]. For biodetection applications, immobilization of biomolecules onto the channel surface is crucial, and in general involves techniques for physical or chemical fixation of the antigen (i.e. cells, proteins) onto a solid support to increase their stability and allow for repeated or continued use [22]. The surfaces of microfluidic devices can be functionalized by adding biotinylated phospholipids to the PDMS prepolymer before curing, enabling protein immobilization on-chip [23]. However, device reuse is not possible when channels are permanently functionalized. For biodetection applications, the selection of suitable materials and methods to facilitate biomolecule immobilization is essential for success.

Core-shelled microspheres are challenging to produce in a controlled manner by mechanical agitation [24] or sonication [25]. Such bulk manufacturing methods lead to polydispersity of particle size, variance in encapsulation efficiency, and stability issues. Membrane emulsification [26] and coaxial flow techniques with acoustic [27] or electrohydrodynamic [28] atomization show promise as new technologies for improving particle size characteristics. However, the synthesis of complex, multiphase and multifunctional particles remains a challenge. Microfluidic flow-focusing based methods [29] provide a high-level of control over reaction conditions, enabling the synthesis of multi-layer lipospheres [30] and polymer microspheres [31,32] with narrow sample size distributions. In this work we combined the elasticity of PDMS with the functional characteristics of polyethyleneglycol (PEG)-lipid derivatives to create a new class of stable and compressible polymer-lipid microbubbles (PLBs) (Fig. 1a). We demonstrate particle trapping within microchannels for biosensing (Fig. 1b) and their use as a template material in the production of 3-D porous structures and surfaces. Incorporating PDMS as a shell component improves the functionality and stability (> 6 months) of the hybrid particles due to the thermally maneuverable solidification process. The use of PDMS as a component in the continuous phase also allows for the creation of porous and functional PDMS microfluidic channel surfaces. With a gas core the particles are compressible and have the potential to respond to acoustic and ultrasound excitations. Furthermore they serve as a non-sacrificial template material for creating porous elastomers, requiring no cumbersome post-processing removal steps. Adding PEG-lipid components within the PDMS shell enables a wide variety of targeting moieties to be incorporated (avidin-biotin, antibodies, DNA, magnetic beads, etc.), allowing for selective targeting. A biocompatible PDMS-lipid-gas composition offers opportunities in creating porous engineered scaffolds, bio-based packaging materials, and delivery (i.e. therapeutic) agents with cell targeting capability.

2. Materials and methods

2.1. Chemicals

Polydimethylsiloxane (PDMS) prepolymer (Sylgard 184) is purchased from K.R. Anderson (Morgan Hill, CA). The stabilizing lipid DSPE-PEG2000-Biotin (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-(Biotinyl(Polyethylene Glycol)2000) and DSPS (1,2-distearoyl-sn-glycero-3-phospho-L-serine) are purchased from Avanti Polar Lipids (Alabaster, AL) as a dry powder. N-Dodecyl β -D-maltoside (DDM), HEPES buffered saline, Hexane, Mineral Oil, and Silicone Oil (Dow Corning Corporation 200 fluid) are purchased from Sigma (St Louis, MO). DiIC18 (1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate), Alexa Fluor® 488 goat anti-mouse IgG, Dulbecco's modified Eagle's medium (DMEM), Fetal Bovine Serum (FBS), and Phosphate-buffered saline (PBS) are purchased from Invitrogen (Carlsbad, CA). EZ-Link Sulfo-NHS-LC-Biotin (sulfosuccinimidyl-6-(biotinamido) hexanoate) and ImmunoPure Avidin are purchased from Pierce (Rockford, IL).

Irregularly shaped Streptavidin coated SPIO particles (BM551, Bangs Laboratories), approximately 2 μ m in diameter, are used to create complexes. The suspension is supplied in phosphate buffered saline (PBS) solution (pH 7.5) containing 0.1% BSA with EDTA and sodium azide added. The original particle concentration is 5 mg mL⁻¹, and is further diluted to 1 mg mL⁻¹. The SPIO particles are >90% in iron oxide composition.

2.2. Particle generation

The microfluidic devices were fabricated from a SU-8 (MicroChem) master with the channels molded in PDMS using standard soft lithography and rapid prototyping techniques [33] (see Supplementary material). A 1% n-Dodecyl β -D-maltoside (DDM) HEPES-buffered saline solution was used without further dilution for the outermost sheath flow stream. For the inner sheath flow stream, PDMS oligomers (20:1 of base and catalyst) were diluted with hexane and low viscosity (4.5 cPs) silicon oil to approximately 70% by weight at a 1:1 ratio of hexane to silicon oil. 50 μ L of the lipid DSPE-PEG2000-Biotin in chloroform was added to the PDMS-solvent mixture at a concentration of 5.0 mg mL⁻¹.

The PDMS microfluidic device was placed on an inverted fluorescence microscope (TE2000, Nikon) and a high-speed camera (Fastcam PCI-10K, Photron Ltd.) is used to capture monochrome still images and record movies. Octafluorocyclobutane (C₄F₈, Scott Specialty Gases) is supplied from a pressurized tank via clear Tygon® tubing and delivered into the gas inlet of the microfluidic chamber using a homemade micro flow meter. The continuous liquid phase mixtures are pumped into the microfluidic device using two digitally controlled syringe pumps (Pico Plus, Harvard Apparatus). A file viewer (PFV, Photron Ltd.) and image analysis program (ImageJ, NIH) are used for data processing and measurements. The polydispersity index $\sigma = \delta/d_{avg} \times 100\%$ was calculated from the average vehicle size d_{avg} and standard deviation δ , determined by measuring the sizes of at least 100 particles from recorded images.

PLBs were trapped within microchannels or allowed to collect within the outlet mineral oil reservoir. A hotplate (Corning) or convection oven at 120°C solidifies the sample in 10 min. Washing the PLBs at 40°C in a 3 mL oil reservoir enables controlled solidification of the particles themselves. The oil reservoir is placed in contact with a temperature-controlled uniformly heated glass slide (TC-GSH, Bioscience Tools) and a stir bar mixes the solution on magnetic-stirrer (Mini-Mag, Cole-Parmer). Attachment to SPIO particles was achieved at the outlet region. Bulk sample temperature is measured with an Extech (Waltham, MA) laser infrared interferometer.

2.3. Labeling and imaging

Approximately 5 μg of the fluorescent probe DiIC18 was added to the initial chloroform lipid suspension and sonicated for 20 min at room temperature. The Alexa Fluor® labeled goat anti-mouse isotype-specific IgG antibodies are supplied as 2 mg mL⁻¹ solutions in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 5 mM sodium azide. For biotinylating the proteins in solution, a 20-fold molar excess of biotin reagent was used to label 1 mg of antibody. The reaction is incubated at room temperature for 30 min. Unlabeled avidin solutions at 10 mg mL⁻¹ were prepared by dissolving the powder in PBS. For PDMS microbubble liposphere binding studies, 10 μl of avidin was added to 500 μl of biotinylated particles. After a 5 min wait time, excess reagents are removed by washing with DI water. A 25 μl solution of antibody was then washed over 1 mL volume of PDMS-lipid microbubbles and rinsed with DI water after a 5 min wait time.

The specimen was imaged with a Zeiss EVO LS 15 environmental scanning electron microscope (Carl Zeiss SMT AG, Germany) using 2.0 kV accelerating voltage at a 10 mm working distance. Fluorescence imaging was performed with an upright microscope (Eclipse E800, Nikon) with Nikon 10 $\times$ NA 0.3 and 40 $\times$ NA 0.75 DIC objectives. The sample was positioned on the stage and illuminated by a 100 W mercury lamp (Chiu Technical Corp) using optical filters for illumination with blue ($\lambda = 475\text{--}490$ nm, FITC) or green ($\lambda = 505\text{--}535$ nm, TRITC) light depending on fluorophore. Detection of green fluorescence occurred at $\lambda = 519$ nm due to Alexa Fluor® 488 labeling and orange-red fluorescence occurred at $\lambda = 569$ nm due to DiI labeling. Images were recorded with a 2-Megapixel color CCD camera (MicroFIRE, Olympus) and processed with PictureFrame 2.0 (Optronics) software.

2.4. Cell culture

HT-1080 human fibrosarcoma-derived cells were donated, and originally obtained from the American Type Culture Collection. Cells were cultured in DMEM containing 10% FBS. PDMS hybrid microbubbles were prepared as described earlier, with the addition of 50 μL DSPPs to the solution at 5 mg mL⁻¹.

3. Results & Discussion

3.1. Design strategies

Multiphase flow in microfluidic systems involves several immiscible fluids separated by flexible fluidic interfaces [34,35]. Our flow-focusing platform (Fig. 2a) is used to spatially organize fluid streams containing various components of our hybrid microbubbles (stabilizer, PDMS-lipid, and gas) and direct them to a focusing region. A shear-induced break-off of fluid streams at the narrowest point (orifice) enables precise control of fluid emulsion sizes [36]. These fluid segments rapidly acquire a spherical shape due to the action of interfacial tension. As flow in these channel networks ($Re < 1$) are affected by viscous and interfacial forces acting on fluid–fluid interfaces, our strategy is to reduce the interplay of these forces that give rise to flow instabilities. Flow rates are adjusted so that the average speed of the liquid fluids ($Q A^{-1}$), where Q is the fluid volumetric flow rate and A is the channel cross-section surface, is similar. Channel lengths were designed to minimize the contact time and angle between the liquid and gas flow streams just prior to the orifice, thereby reducing viscous instabilities. This also minimizes gas diffusion through the elastomeric polymer.

A HEPES-buffered saline solution containing 1% n-Dodecyl β -D-maltoside (DDM) served as the outermost flow stream. As a non-ionic surfactant, DDM has dual hydrophobic/hydrophilic properties that allow lipid displacement, while still providing coverage on the native lipid-modified PDMS shell. Considerable reduction in pre-cure PDMS (Sylgard 184) viscosity (3900 cPs) to a range between 100–500 cPs was necessary prior to use of the mixture as the primary

continuous phase within microchannels. PDMS oligomers were diluted with hexane and low viscosity (4.5 cPs) silicon oil at equal ratios to approximately 70% by weight. Dilution with hexane produced the least viscous solution, but 4.5 cPs silicon oil produced a fast cure time while still providing a workable solution during the experiment time of 2 hrs. The final combination (160 cPs) was empirically determined. Due to chloroform solubility in hexane, approximately 50 μL of the lipid 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-(Biotinyl(PolyethyleneGlycol)2000) (DSPE-PEG2000-Biotin) was added to the solvent mixture at a concentration of 5.0 mg mL^{-1} . The diluted PDMS-lipid mixture served as the inner sheath flow stream. The stabilizing gas octafluorocyclobutane (C_4F_8) was used as the dispersed phase. The diameter does not change significantly for these vehicles as they are produced at the device orifice (Fig. 2b) and rise from the outlet due to buoyancy (Fig. 2c).

3.2. Characterization of polymer-lipid microbubbles

Stable production rates of 1.5×10^3 PLBs per minute have been achieved with runtimes lasting 1–2 hours. While flow-focusing is rather slow at generating microbubbles for contrast-enhanced biomedical imaging applications [37], the production rate achieved here is sufficient for on-chip immunoassay or microarray applications where a single PLB contains multiple binding sites. PLBs are visible in brightfield, collected on the cover glass after approximately 30 min of 40°C heat treatment. The outer PDMS-lipid shell with DMM (Fig. 2d) stabilizes the vehicle, and incorporates biotin-PEG for biosensing and targeting. With a hollow gas core (Fig. 2e) the particles are compressible. The orange-red fluorescence of DiIC18 (Fig. 2f), a lipophilic membrane stain that is weakly fluorescent until incorporated into membranes, confirms the lipid coating within the PDMS shell.

The average size of PLBs can be increased by increasing the gas flow rate with respect to the PDMS-lipid and DDM-HEPES buffered saline solution flow rates. The continuous phase PDMS-lipid flow increases the viscosity of the bulk solution and reduced DDM-HEPES buffered saline solution flow increases the interfacial tension of the shell, leading to larger and more dense PLBs that have a higher polydispersity. The shell-thickness can be tuned by adjusting the PDMS-lipid flow rate with respect to the DDM-HEPES buffered saline solution flow rate. The smaller sized PLBs ($\sim 10 \mu\text{m}$) were produced at a PDMS-lipid mixture flow rate of 3 $\mu\text{L min}^{-1}$, DDM-HEPES buffered saline solution flow rate of 7 $\mu\text{L min}^{-1}$, and a PFC gas pressure of 1.0 psi gauge. The larger sized PLBs ($\sim 35 \mu\text{m}$) were produced at a PDMS-lipid mixture flow rate of 3.0 $\mu\text{L min}^{-1}$, DDM-HEPES buffered saline solution flow rate of 7.0 $\mu\text{L min}^{-1}$, and a PFC gas pressure of 3.0 psi gauge. PLBs have good monodispersity ($\sigma < 10\%$) across three different population sizes as shown in Figure 3. As a novel preparation technique, flow-focusing microfluidic devices [38] provide a new means for the synthesis of advanced materials with tailored properties.

Microfluidic channel geometries can be designed based on flow resistances to trap and hold particles in place while fluid continues to move [39]. Two sizes of PLBs (10 μm and 45 μm) were captured and immobilized (Fig. 4a,b) in microfluidic traps to demonstrate potential biosensing applications on-chip. When using 4.5 cPs silicon oil without further dilution, heating the sample suspension in a convection oven at 120°C for 10 min solidifies the entire sample, embedding the microbubbles within the polymer matrix (Fig. 4c). Spreading this outlet solution with a glass stir rod and subsequent heating allows us to create thin ($\sim 50 \mu\text{m}$ thick) porous elastomeric membranes (Fig. 4d). These pores become a permanent feature of the polymer matrix (Fig. 4e). After thermal solidification, they are size-stable for more than six months of monitoring.

The packing density can largely be controlled based on production rate, resulting in densely packed PLB clusters that are visually observed by SEM (Fig. 5a). The PLBs have a smooth surface and the hollow interior is evident. Having the sample continuously flow into a heated

mineral oil bath enables controlled solidification of the particles themselves. PLBs become rapidly embedded in the matrix in a matter of seconds at 120°C (Fig. 5b) without continuous stirring of the mineral oil during the solidification process. With 40°C curing and continuous stirring, we are able to obtain free floating single layer PLBs (Fig. 5c).

3.3. Applications

DSPE-PEG2000-Biotin was chosen to test the functionalization of the PDMS microbubbles, and potential for biosensing applications. Similar PEG-lipid derivatives have been used to prolong the *in-vivo* circulation time of liposomes and to evaluate steric stabilization of amphiphathic polymers [40]. After observing clusters in brightfield (Fig. 6a), fluorescence imaging with biotinylated Alexa Fluor 488 labeled goat anti-mouse isotype-specific IgG antibodies is performed to visualize the shell surface. DDM has been previously shown to minimize nonspecific protein adsorption on PDMS surfaces to the single molecule level [41]. Here, the inhomogeneity of the bright green fluorescence (Fig. 6b) indicates that DDM may restrict protein adsorption on the PLB shell. More importantly, these results indicate that biotin groups are present on the outer PLB shell surface after PEG-lipid modification, and their presence allows avidin binding. No decrease in shell surface fluorescence intensity is observed after sample washing, or after a few days at STP conditions.

Streptavidin-bound superparamagnetic iron oxide particles can be attached to the biotinylated lipid of the PDMS shell (Fig. 6c). Streptavidin is covalently attached to the SPIO surface, with each molecule providing four binding sites for the biotin groups of the PLB shell. The magnetic field gradient exerts a force on the SPIO particles, resulting in movement of the PLBs and allowing for sorting and precise positioning at desired locations. If in close proximity, PLBs can be arranged to link together after heat treatment to form a chain of particles.

Attachment and uptake of PLBs with HT-1080 fibrosarcoma cells was done in the presence of serum, underneath a cover slip. Cells were found to favorably attach to < 15 μm PLBs (Fig. 6d). HT-1080 cells and other phagocytes are known for recognizing and engulfing targets that express surface phosphatidylserine (PS) such as apoptotic cells [42]. We found that incorporation of DSPS enhanced the ability of the HT-1080 cells to bind to the PLBs. The cells would otherwise clump together in clusters. It is possible that the serum provides additional molecules to promote adhesion, or may stimulate uptake by phagocytes. Upon cell attachment, PLBs dissolve quickly within 5 – 10 sec, monitored by light microscopy. Unattached PLBs were stable throughout the experimental period.

4. Summary

We have synthesized long-lasting polymer-lipid microbubbles (PLBs) with a microfluidic method and have demonstrated their capability in biodetection and the creation of porous structures. Microfluidic flow-focusing based methods allow for the control of interior gas composition as well as shell composition and shell thickness. The shell-thickness can be tuned by adjusting fluid flow-rates. After thermal solidification, they are highly size-stable and have low polydispersity values ($\sigma < 10\%$). Stability has been demonstrated for a period of more than six months, compared with minutes to hours in current lipid-shelled microbubble systems. By trapping the particles on-chip, multiple detection schemes may be incorporated based on the chemistry of the PEG-lipid derivatives in the PDMS shell.

With a densely packed arrangement, we foresee the use of PLBs at the point -of-care as antibody/protein and DNA/RNA binding agents in immunoassays and microarrays, and as bio-based packaging materials. Potential *in-vivo* applications exist for use as porous engineered scaffolds, as injectables for diagnostics with medical imaging modalities (i.e. US, MRI), and

as delivery (i.e. therapeutic) agents that target cells and allow for the controlled release of substances, which we intend to explore in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the National Institutes of Health (NIH Grant # R01 EB008733) for financial support. This work used instrumentation at the Carl Zeiss Center of Excellence for Electron Microscopy at UCI.

References

1. Dendukuri D, Doyle PS. *Adv Mater* 2009;21:1–16.
2. Leong-Poi H, Christiansen J, Heppner P, Lewis CW, Klivanov AL, Kaul S, Lindner JR. *Circulation* 2005;111:3248–3254. [PubMed: 15956135]
3. Ferrara K, Pollard R, Borden M. *Annu Rev Biomed Eng* 2007;9:415–447. [PubMed: 17651012]
4. Peng SL, Zhang MY, Niu XZ, Wen WJ, Sheng P, Liu ZY, Shi J. *Appl Phys Lett* 2008;92 012108.
5. Wan J, Bick A, Sullivan M, Stone HA. *Adv Mater* 2008;20:3314–3318.
6. van der Net A, Delaney GW, Drenckhan W, Weaire D, Hutzler S. *Colloid Surface A* 2007;309:117–124.
7. Pancrazio JJ, Whelan JP, Borkholder DA, Ma W, Stenger DA. *Ann Biomed Eng* 1999;27:697–711. [PubMed: 10625143]
8. Hettiarachchi K, Talu E, Longo ML, Dayton PA, Lee AP. *Lab Chip* 2007;7:463–468. [PubMed: 17389962]
9. Park JJ, Nie Z, Kumachev A, Abdelrahman AI, Binks BR, Stone HA, Kumacheva E. *Angew Chem Int Edit* 2009;48:5300–5304.
10. Broyles BS, Jacobson SC, Ramsey JM. *Anal Chem* 2003;75:2761–2767. [PubMed: 12948147]
11. Dittrich PS, Manz A. *Nat Rev Drug Discov* 2006;5:210–218. [PubMed: 16518374]
12. Gordillo JM, Cheng Z, Ganan-Calvo AM, Márquez M, Weitz DA. *Phys Fluids* 2004;16:2828–2834.
13. Thorsen T, Roberts RW, Arnold FH, Quake SR. *Phys Rev Lett* 2001;86:4163–4166. [PubMed: 11328121]
14. Lee CC, Sui GD, Elizarov A, Shu CYJ, Shin YS, Dooley AN, Huang J, Daridon A, Wyatt P, Stout D, Kolb HC, Witte ON, Satyamurthy N, Heath JR, Phelps ME, Quake SR, Tseng HR. *Science* 2005;310:1793–1796. [PubMed: 16357255]
15. Whitesides GM. *Nature* 2006;442:368–373. [PubMed: 16871203]
16. Juchniewicz M, Stadnik D, Biesiada K, Olszyna A, Chudy M, Brzozka Z, Dybko A. *Sensor Actuat B-Chem* 2007;126:68–72.
17. Dufaud O, Favre E, Sadtler V. *J Appl Polym Sci* 2002;83:967–971.
18. Moorthy J, Beebe DJ. *Lab Chip* 2003;3:62–66. [PubMed: 15100783]
19. Lagally ET, Scherer JR, Blazej RG, Toriello NM, Diep BA, Ramchandani M, Sensabaugh GF, Riley LW, Mathies RA. *Anal Chem* 2004;76:3162–3170. [PubMed: 15167797]
20. Ottesen EA, Hong JW, Quake SR, Leadbetter JR. *Science* 2006;314:1464–1467. [PubMed: 17138901]
21. Zaytseva NV, Montagna RA, Baeumner AJ. *Anal Chem* 2005;77:7520–7527. [PubMed: 16316157]
22. Chandrasekaran A, Acharya A, You JL, Soo KY, Packirisamy M, Stiharu I, Darveau A. *Sensors* 2007;7:1901–1915.
23. Huang B, Wu HK, Kim S, Kobilka BK, Zare RN. *Lab Chip* 2006;6:369–373. [PubMed: 16511619]
24. Tartis MS, McCallan J, Lum AF, LaBell R, Stieger SM, Matsunaga TO, Ferrara KW. *Ultrasound Med Biol* 2006;32:1771–1780. [PubMed: 17112963]
25. Fang JY, Hung CF, Liao MH, Chien CC. *Eur J Pharmaceut Biopharmaceut* 2007;67:67–75.

26. Wang LY, Ma GH, Su ZG. *J Control Release* 2005;106:62–75. [PubMed: 15922472]
27. Berkland C, Pollauf E, Pack DW, Kim K. *J Control Release* 2004;96:101–111. [PubMed: 15063033]
28. Stride E, Edirisinghe M. *Soft Matter* 2008;4:2350–2359.
29. Xu S, Nie Z, Seo M, Lewis P, Kumacheva E, Stone HA, Garstecki P, Weibel DB, Gitlin I, Whitesides GM. *Angew Chem Int Ed Engl* 2005;44:724–728. [PubMed: 15612064]
30. Hettiarachchi K, Zhang S, Feingold S, Lee AP, Dayton PA. *Biotechnol Prog* 2009;25:938–945. [PubMed: 19455647]
31. Liu L, Yang JP, Ju XJ, Xie R, Yang LH, Liang B, Chu LY. *J Colloid Interface Sci* 2009;336:100–106. [PubMed: 19394626]
32. Shum HC, Kim JW, Weitz DA. *J Am Chem Soc* 2008;130:9543–9549. [PubMed: 18576631]
33. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu HK, Schueller OJA, Whitesides GM. *Electrophoresis* 2000;21:27–40. [PubMed: 10634468]
34. Gunther A, Jensen KF. *Lab Chip* 2006;6:1487–1503. [PubMed: 17203152]
35. Shui L, Eijkel JCT, van den Berg A. *Adv Colloid Interfac* 2007;133:35–49.
36. Tan YC, Hettiarachchi K, Siu M, Pan YR, Lee AP. *J Am Chem Soc* 2006;128:5656–5658. [PubMed: 16637631]
37. Feshitan JA, Chen CC, Kwan JJ, Borden MA. *J Colloid Interface Sci* 2009;329:316–324. [PubMed: 18950786]
38. Stride E, Edirisinghe M. *Med Biol Eng Comput* 2009;47:883–892. [PubMed: 19434435]
39. Tan WH, Takeuchi S. *P Natl Acad Sci* 2007;104:1146–1151.
40. Mori A, Klibanov AL, Torchilin VP, Huang L. *Febs Lett* 1991;284:263–266. [PubMed: 2060647]
41. Huang B, Wu HK, Kim S, Zare RN. *Lab Chip* 2005;5:1005–1007. [PubMed: 16175253]
42. Fadok VA, de Cathelineau A, Daleke DL, Henson PM, Bratton DL. *J Biol Chem* 2001;276:1071–1077. [PubMed: 10986279]

Fig. 1. Schematics and applications. (a) Hybrid polymer-lipid functionalized microbubble. The outer PDMS-lipid shell stabilizes the vehicle and the gas interior makes the vehicle compressible. (b) Cured spherical PLBs can be densely or loosely packed within porous elastic membrane for biosensing. B, S, A, and F correspond to biotin, Streptavidin, antibody, and fluorophore respectively.

Fig. 2.

Geometry of device for PLB production with generated particles. (a) All channels have a rectangular cross section and a height of $45\ \mu\text{m}$. The widths of the outer solvent, lipid, and gas inlet channels are $\{20, 30, 20\ \mu\text{m}\}$ respectively. The device features an expanding nozzle with an orifice width of $20\ \mu\text{m}$. The particles are cured in a temperature controlled oil bath or an oven. (b) PLB generation at the orifice. (c) Moving within channel and rising to the top of the outlet. (d) Outer PDMS shell, (e) gas core, and (f) DiI fluorescence within the shell, confirming lipid within the PDMS shell.

Fig. 3.
Size distribution of PLBs with frequency as a percentage across three populations.

Fig. 4. Trapped PLBs on-chip and within porous elastomers. (a) Brightfield images of 10 μm and (b) 45 μm PLBs trapped within microchannels of a microfluidic device. Fluid continues to flow. (c) PLBs embedded within PDMS elastomer matrix after a 120°C heat treatment. (d) SEM image of 50 μm thick porous elastomeric membrane, and (e) magnified cross-section containing micron-sized holes.

Fig. 5. Microscopy images of particles. (a) SEM image of densely packed PLBs, bound to a thin elastomer layer. (b) PLBs embedded together in the PDMS bulk continuous phase without stirring. (c) Free-floating PLBs isolated and separated from the PDMS carrier fluid.

Fig. 6. Antibody binding and other applications. (a) Brightfield image of PLBs after a 120°C heat treatment and (b) fluorescence confirming IgG antibody binding to functionalized outer PLB shell. (c) Brightfield image of a 30 µm PLB with brown irregular shaped SPIO particles (~2 µm) attached to the outer shell. (d) Brightfield image of HT-1080 cell attachment on a 15 µm PLB.