Supplementary Data

Device Fabrication Details

There were three primary pieces for each bioreactor. A front plate served to close the system and to provide holes for the addition of filling and emptying ports by tapping 1/4″-28 holes and connecting to screw-to-Luer Lock Quick Turn Couplings (McMaster-Carr, Elmhurst, IL). The front plate of the novel bioreactors contained an additional port at the terminal end of the channel fitted with a pressure-activated Luer valve for filling and bubble removal. A central acrylic ring served as the perimeter wall for the fluid, with the novel variants containing the bubble catch channel. The back wall served as a vented backing for gas perfusion and as an anchor point for attachment to a rotation system through a 5/8″-11 nylon nut (McMaster-Carr). All acrylic pieces were precut to accept M4 bolt holes around their perimeter for assembly. After taping to add thread, all acrylic pieces were annealed in an 85°C oven and gradually cooled to room temperature. Finally, the pieces were assembled in the following order: front acrylic plate, silicone gasket, central acrylic ring, silicone gasket, gas-permeable membrane (Tegaderm facing central ring), back acrylic plate, and nylon nut. The assembled bioreactor was held together with M4x16 bolts with two flat washers, one spring washer, and one nut each.

Device Dimensions

Common dimensions

Acrylic thickness: 4.2 mm
Gasket silicone thickness: 0.5 mm
Membrane thickness: 0.2 mm
Coupling nut type: 5/8″-11
Total thickness: 14.5 mm
Main volume diameter: 50 mm

Standard bioreactor specifications

Total volume: 10 mL
Outer diameter: 76 mm

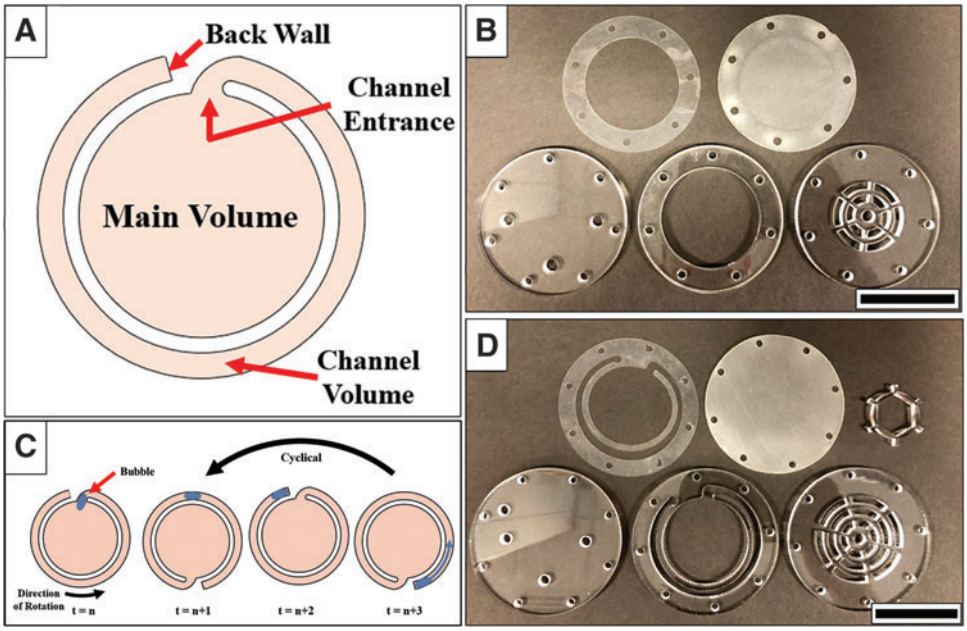
Bubble-catching bioreactor specifications

Total volume: 13 mL
Main chamber volume: 10 mL
Channel volume: 3 mL
Interior diameter (channel): 66 mm (channel diameter: 3.8 mm)
Channel entrance width: 8 mm
Channel wall thickness: 3 mm
Outer diameter: 87 mm

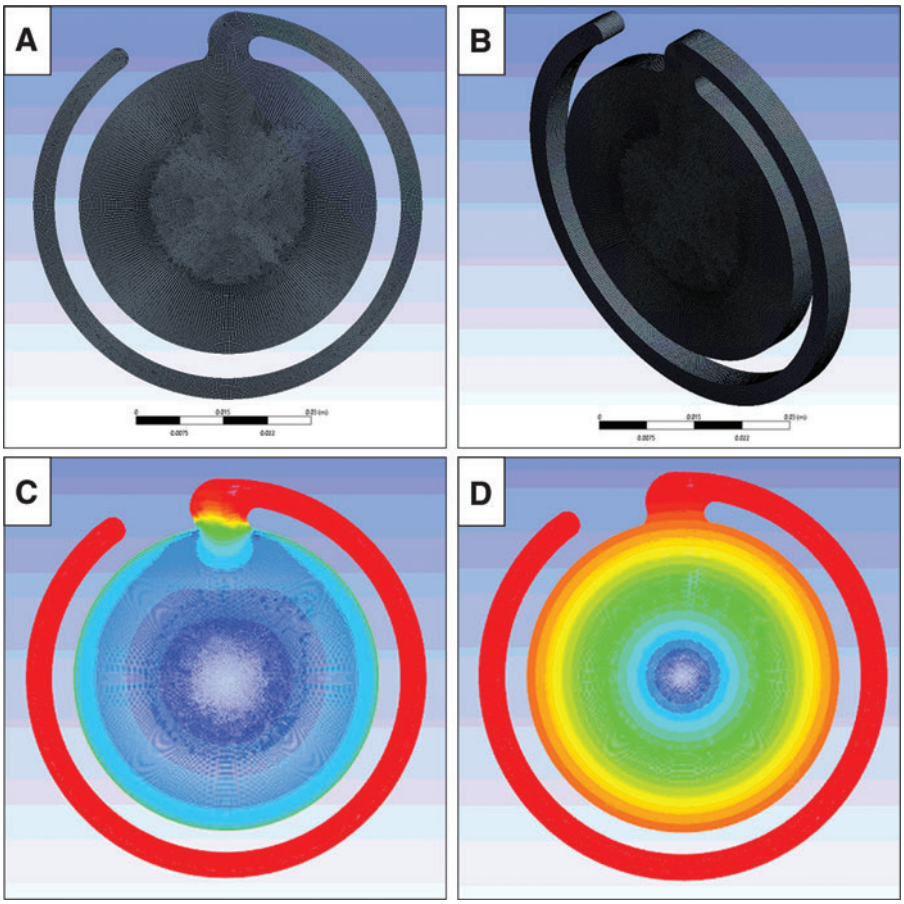
Computational Fluid Dynamics Details, Boundary Conditions, and Settings

For models not containing bubbles, the following settings were used

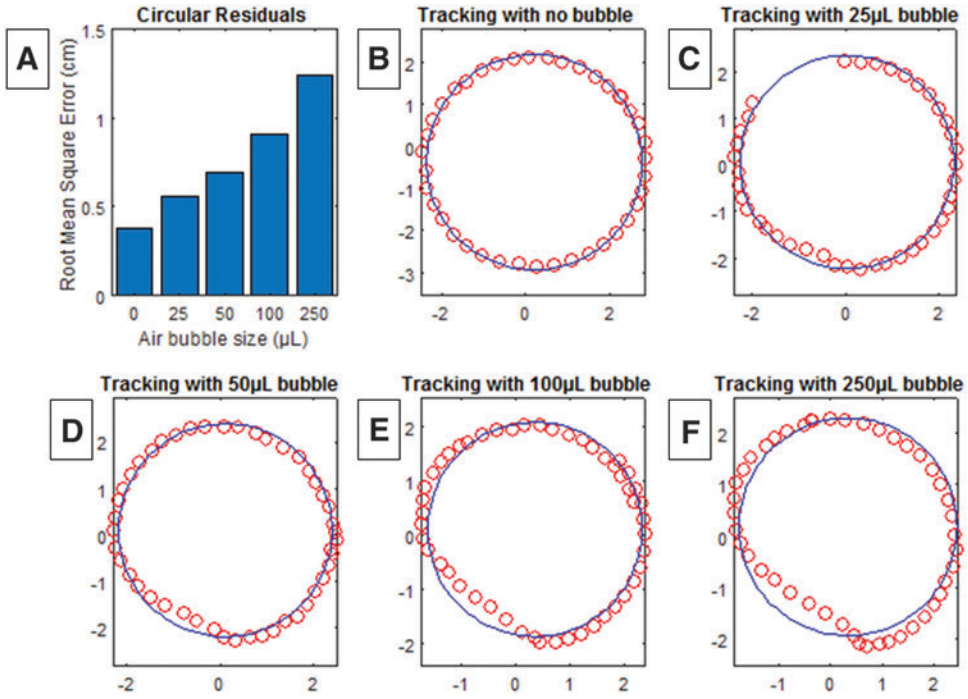
Solver: type = pressure-based
Model: multiphase = off, viscous = laminar, energy = off (incompressible).
Shear condition: no slip



SUPPLEMENTARY FIG. S1. Design and fabrication of the bioreactors. **(A)** A description of key parts of the novel BCB design. **(B)** The laser-cut components of the replicated standard design. **(C)** A description of how bubbles are captured and isolated in the novel BCB. **(D)** The laser-cut components of the novel BCB design. Scale bars are 5 cm. BCB, bubble-capturing bioreactor.



SUPPLEMENTARY FIG. S2. Set of grid-independent validations performed on the modified, bubble-capturing design at a node size of 0.325 mm. **(A)** A front-facing view of the CFD mesh in the novel BCB. **(B)** An isometric view of the mesh. **(C)** The CFD fluid velocity vectors after 0.01 s. **(D)** The fluid velocity vectors after stabilization. All scale bars are 3 cm. CFD, computational fluid dynamics.



SUPPLEMENTARY FIG. S3. Dose-dependent effects of bubbles on bioreactor behavior. **(A)** The circular residuals of tracked alginate particle motion to represent the degree of deflection when encountering bubbles of various sizes. **(B–F)** Graphing representation of the tracked alginate particle data fit against a circle to obtain residuals.

Supplementary Table S1. All Maximum and Average Shear Values—A List of the Maximum and Average Shear Values Obtained from the Alginate Bead Shear Experiments

	Max shear (dynes/cm^2)	*Average shear (dynes/cm^2)*
BCB: no bubble	0.098 ± 0.007	0.036 ± 0.003
BCB: 500 μL bubble	0.098 ± 0.017	0.048 ± 0.007
Standard: no bubble	0.082 ± 0.019	0.036 ± 0.002
Standard: 25 μL bubble	0.183 ± 0.024	0.081 ± 0.010
Standard: 50 μL bubble	0.215 ± 0.016	0.100 ± 0.016
Standard: 100 μL bubble	0.244 ± 0.033	0.119 ± 0.011
Standard: 250 μL bubble	0.350 ± 0.058	0.159 ± 0.015
Standard: 500 μL bubble	0.382 ± 0.027	0.119 ± 0.002

Plus/minus values indicate standard deviation. All experiments were performed with $n = 3$.
BCB, bubble-capturing bioreactor.

Fluid: water (default), density = 998.2 kg/m^3, viscosity = 0.001003 kg/m^{-s}
3D depth of the design: 4.8 mm

For models containing bubbles, the following settings were used

Solver: type = pressure-based
Model: multiphase = eulerian (phase interaction—wall lubrication = antal-et-al, surface tension = 0.07, viscous = laminar, energy = off (incompressible).
Shear condition: no slip
Fluid (phase 1): water (default), density = 998.2 kg/m^3, Viscosity = 0.001003 kg/m^{-s}
Fluid (phase 2): water-vapor (default), density = 0.5542 kg/m^3, Viscosity = 1.34e^{-05} kg/m^{-s}
3D depth of the design: 4.8 mm

Simulations were run at 1 G and at a rotational speed of 10 rpm. The shown computed results were obtained at a node length of 1.0 mm and a time step of 0.01 s. Grid- and time-independent analyses were performed by verifying the observed dynamics at a node size of 0.325 mm (~500,000 nodes per simulation) and a time step of 0.005 s, respectively (Supplementary Fig. S1). All four computational fluid dynamics (CFD) simulation conditions were also run with the laminar viscous model substituted for the k-epsilon (2 eqn) model with no observable change in results. Both nonbubble conditions were run with the Eulerian multiphase model, described above, with no observable difference in the results from multiphase: off. Additionally, both bubble-containing simulations were run with both water vapor bubbles and dry air bubbles. The changes in the type of gaseous material did not yield observable differences in the results. Finally, all four conditions were run with a Volume of Fluid multiphase model. For the ''no-bubble'' conditions, there was no observable change. For the ''with bubble'' conditions, the shapes of the gas bubbles less closely resembled the empirical observations than the Eulerian model. Irrespective of the close similarity between empirical observations and numerical prediction, the Eulerian model was chosen for our simulations to assess the probability of a bubble splitting or frothing when encountering the edge of the channel wall while entering the channel in the novel design, of which, none was observed. Water vapor was chosen over dry air to more closely simulate the very high humidity environment of incubators and subsequently bubbles that form at liquid interfaces in them. Preliminary results indicated that performing the simulations at various speeds from 5 rpm to 25 rpm did not affect the calculated relative velocities or fluid paths (not shown).

Alginate Bead Experiment Details

Alginate beads were produced by dropwise adding a (w/v) aqueous mix of 0.75% sodium alginate (Sigma-Aldrich, St. Louis, MO), 1% polyethylene oxide (Sigma-Aldrich), and 2% powdered charcoal (for color) into a stirred 2% calcium chloride bath. All shear values obtained are reported in Supplementary Table 1.

Explanation of the Circularity Equation for Figure 3

The circularity value reported in Figure 3H is an output value using the ImageJ ''Circularity'' function. After each spheroid had its perimeter traced, the software automatically calculated the total perimeter and total area of each image. The circularity function outputs a value according to the equation below:

$$circularity = 4\pi\left(area/perimeter^2\right)$$

This generates a value between 0 and 1, which gives an estimate of how circular a two-dimensional object is. We chose to report this value to demonstrate that the modified design did not substantially change spheroid morphology. Had this been the case, spheroids may have been observed

Supplementary Table S2. A Description of Cell Experiment Conditions—A Detailed Description of the Four Primary Conditions Used in the Cell Culture Experiments Described in Primary Figure 3

	Novel BCB without bubble	*Novel BCB with bubble*	*Standard HARV-type without bubble*	*Standard HARV-type with bubble*
Bubble size	0 μL	300 μL	0 μL	300 μL
Initial cell density	100,000 cells/mL	100,000 cells/mL	100,000 cells/mL	100,000 cells/mL
Rotational speed	10 rpm	10 rpm	10 rpm	10 rpm
Culture time	72 h	72 h	72 h	72 h
n	10	10	8	11
No. rejected	0	0	5	0

HARV, high aspect ratio vessel.

as significantly ellipsoid from spinning on their longer axis for a higher percentage of the time, aggregating into larger, amorphous shapes, or being torn into random, pointed shapes. Our data suggest none of these is the case.

Cell Culture Details

To sterilize and condition the bioreactors, each was filled with 0.2 N NaOH for 24 h. All bioreactors were then moved to a laminar flow hood and exposed to UV sterilization for 30 min on each side. They were then emptied of the NaOH and rinsed three times with sterile 1 × phosphate-buffered saline (PBS) (Corning, Corning, NY). Next, the bioreactors were filled with a sterile conditioning solution composed of 10% fetal bovine serum (FBS) (Gibco, Gaithersburg, MD), 88% 1 × PBS (Corning), and 2% Penicillin/Streptomycin (Pen-Strep; Gibco) and incubated at 37°C for 24 h while rotating at 10 rpm on a 4RCCS rotary system (Synthecon, Inc., Houston, TX). After conditioning, the bioreactors were emptied aseptically and immediately used for cell culture. Cells were maintained in a T75 flask (Falcon) in Dulbecco's Modified Eagle Medium (DMEM) (Gibco) supplemented with 10% FBS, 1% Pen-Strep, and 1% L-glutamine (Gibco). Once the cultures reached ~80–90% confluence, cells were rinsed with PBS and detached using 1 × Trypsin (Gibco), and resuspended in DMEM at a concentration of 1×10^6 cells/mL. A brief description of experimental conditions is shown in Supplementary Table 2.