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Rotating-liquid-based hydrogel bead generator

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ABSTRACT

Hydrogel beads are widely used in various applications, but producing such beads often requires complicated devices. Instead, we propose an easy-to-adopt, cost effective, open source hydrogel bead generator. This generator consists of two modules. The first module rotates two immiscible liquids in rigid body motion: mineral oil as the continuous phase (CP) liquid on top, and a hydrogel cross-linking (CL) liquid at bottom. The second module injects a hydrogel pre-polymer solution as the dispersed phase (DP) liquid into the rotating CP liquid. As the DP liquid flows out of a syringe needle, its drops are pinched off by the shear force from the CP liquid, and move with the CP liquid while settling down. When the drops enter the CL liquid, they become hydrogel beads. Experiments using water and mineral oil showed that the size of produced drops could be controlled by adjusting the flow speed of the CP and DP liquids. A demonstration using alginate showed that the proposed generator could successfully create alginate gel beads of uniform size and shape.

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1. Hardware in context

Hydrogel beads of uniform size and shape are useful in various fields including pharmaceutical, cosmetic, and food industries [1]. The most common method to produce such hydrogel beads is to create monodisperse drops of the pre-polymer solution of a hydrogel, and then to gelate the drops using a cross-linker. Thus, a crucial step in this process is typically producing monodisperse drops. For this purpose, various drop generation methods had been developed. Currently the most widely used drop generation method in research settings is microfluidic drop generators [1–5]. In the microfluidic drop generator, microchannels guide the flows of continuous phase (CP) and dispersed phase (DP) liquids that are injected into the device separately, and drops are generated at the junction of the channels where the CP and DP liquids come into contact at a certain angle. Various designs of the microfluidic drop generator, such as T-junction, have been developed depending on applications. Such microfluidic drop generators can be used to produce hydrogel beads by injecting a pre-polymer solution into the device as the DP liquid and inducing cross-linking of created drops in the device [6,7].

Typically, a microfluidic gel bead generator is made of polydimethylsiloxane (PDMS), and its fabrication process in a laboratory requires a master mold having microchannel patterns in a high resolution. Such a microfluidic mold is fabricated using photolithography, which requires a high-resolution photo mask, a mask aligner with an ultraviolet (UV) light, a spin coater, and so on. Additionally, photolithography is usually done in a clean room or similar environments. Therefore, photolithography can be a hurdle to fabricate microfluidic gel bead generators in a certain research laboratory. Once fabricated, moreover, the microfluidic channel geometry, which determines how CP and DP flows interact, cannot be changed. Thus,

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new devices need to be fabricated when different channel designs are needed, and the degree of freedom for *in situ* adjustment of drop generation is limited although it is possible to adjust the flow speed of CP and DP liquids for various drop sizes.

This paper describes an open source rotating-liquid-based hydrogel bead generator. This setup continuously generated alginate solution drops (DP) in mineral oil (CP) rotating in rigid body motion. The drop generation principle of the proposed method is similar to that of the T-junction microfluidic drop generator and other cross-flow-based drop generators: DP drops were sheared off by the cross-flow of the CP liquid. Created DP drops settled down in the DP liquid and underwent gelation upon reaching the calcium ion solution (cross-linking [CL] liquid) beneath the CP liquid layer. As the final product, alginate beads of uniform size and shape were collected from the CL liquid. The major advantage of the presented gel bead generator is its ease of assembly and maintenance because it consists of commercially available parts and each part is replaceable. In addition, the parameters related to drop generation, including the flow speeds of CP and DP liquids, can be easily changed to control the size of created drops and thus to produce hydrogel beads of various sizes.

2. Hardware description

The presented hydrogel bead generator mainly consists of two modules: one for rotating a container, which contains a CP liquid and a CL liquid, at a constant rotational speed, and the other for injecting a DP liquid into the rotating CP liquid (Fig. 1). The former consists of a commercial turntable with the center shaft removed, a cylindrical container, and the CP and CL liquids. The latter consists of a syringe needle with the tip immersed in the CP liquid, a needle holder fixed to a linear stage installed on a frame, a syringe pump with tubing, and a DP liquid. Building this setup is cost effective because materials can be easily found, purchased, and even replaced with more affordable options. In addition, the setup enables easy maintenance and modification because every part can be disassembled, replaced, and even customized.

The hydrogel bead generator is operated in the following order: (1) the CL liquid and the CP liquid are sequentially poured into the container, (2) a syringe needle of a desired inner diameter is installed on the syringe holder, (3) the syringe needle holder is moved to a desired location with respect to the axis of rotation of the container, (4) the rotational speed of the turntable is set, and the turntable is turned on, so that the CP liquid rotates at a constant rotational speed in rigid body motion, (5) the syringe pump is set to a desired volume flow rate and then turned on, (6) the DP liquid is injected into the rotating CP liquid, and created DP drops fall down towards the CL liquid by gravity, (7) once drops become hydrogel beads entering the CL liquid, the syringe pump and the turntable are turned off sequentially, and (8) hydrogel beads are harvested from the bottom of the container.

The diameter and generation frequency of drops and thus hydrogel beads can be controlled by changing the average speed of the DP flow through the needle (V_d), and the linear speed of the CP liquid motion at the needle tip (V_c). The former is determined by the inner diameter of the syringe needle (D_i) and the injection volume flow rate of the syringe pump (Q): $V_d = 4Q/(\pi D_i^2)$, and the latter by the distance from the needle to the axis of rotation of the container (R), and the rotational speed of the CP liquid (ω): $V_c = R\omega$. Thus, it is possible to modulate V_d and V_c by changing D_i and Q , and R and ω , respectively.

In summary, this rotating liquid-based hydrogel bead generator can be used with the following benefits:

- Low cost and easiness of system building.
- Easy observation of drop or gel bead generation.
- Easy maintenance, modification and part replacement.
- Friendly for users without prior experiences.
- Open source hardware for further customizing.

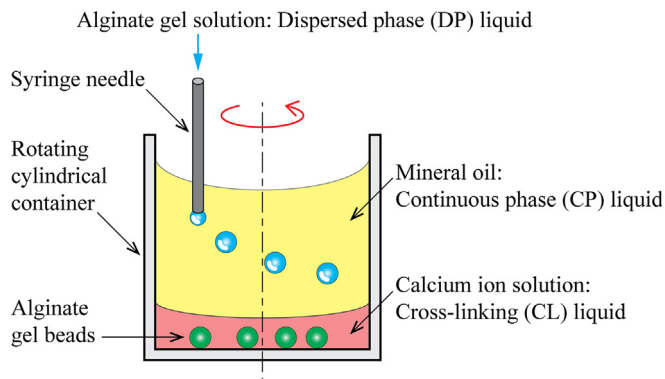


Fig. 1. Schematic figure of the rotating-liquid-based hydrogel bead generator.

3. Bill of materials

Designator	Component	Number	Cost per unit -USD	Total cost - USD	Source of materials	Material type
A1	Pioneer Belt Drive Stereo Turntable, PL-112D	1	295	295	www.ebay.com	Non-specific
A2	Imagitarium, Freshwater Aquarium, 2.2 gallon	1	27.99	27.99	www.amazon.com	Non-specific
B0	Actobotics® Hardware Pack A (632146)	1	39.99	39.99	www.servocity.com	Metal
B1	Side Tapped Pattern Mount C (545360)	2	5.99	11.98	www.servocity.com	Metal
B2	1.50" Aluminum Channel (585440)	1	2.99	2.99	www.servocity.com	Metal
B3	Mini V-Wheel Standoff (4 ea/pack) (633176)	2	4.99	9.98	www.servocity.com	Metal
B4	Mini V-Wheels (2 ea/pack) (615454)	4	6.99	27.96	www.servocity.com	Other
B5	6-32 Button Head Hex Drive Screws 5/16" (97763A403)	8	0.15	1.2	www.servocity.com	Metal
B6	8 mm Lead Screws 25.6" (650 mm) (3501-0804-0650)	1	13.99	13.99	www.servocity.com	Metal
B7	8 mm Lead Screw Nut, 0.770" Pattern (545315)	4	7.99	31.96	www.servocity.com	Metal
B8	Actobotics X-Rail® 24" (565074)	10	8.57	85.7	www.servocity.com	Metal
B9	90° Single Angle Short Pattern Bracket (585506)	4	1.99	7.96	www.servocity.com	Metal
B10	X-Rail Screw Plate (2 ea/pack) (585757)	26	4.99	129.74	www.servocity.com	Metal
B11	4.5"×1.5" Pattern Plate (5 hole) (585722)	2	2.39	4.78	www.servocity.com	Metal
B12	Actobotics X-Rail® 18.00" (565062)	4	7.25	29	www.servocity.com	Metal
B13	X-Rail L-Bracket (2 ea/pack) (585076)	2	1.99	3.98	www.servocity.com	Metal
B14	Flush Perpendicular X-Rail Mount (585073)	12	3.99	47.88	www.servocity.com	Metal
B15	X-Rail T-Bracket (2 ea/pack) (585075)	6	1.99	11.94	www.servocity.com	Metal
C1	BD 3 mL Luer-Lok Tip Syringe (200 ea/pack)	1	33.25	33.25	www.fishersci.com	Other
C2	Actobotics X-Rail® 15.00" (565054)	1	5.93	5.93	www.servocity.com	Metal
C3	Cole-Parmer Tygon Tubing, 0.020" ×0.060"OD (100 ft/roll)	1	57.2	57.2	www.coleparmer.com	Other
C4	Gorilla 2 Part Epoxy (Clear)	1	4.97	4.97	www.amazon.com	Other
C5	SAI 23G 0.5" Blunt Needle (100 ea/pack)	1	18.57	18.57	www.sai-infusion.com	Other
O1	Scotch Double Sided Tape	1	3.29	3.29	www.amazon.com	Other
O2	iCraft PeelnStick Removable Ruler Tape	1	5.5	5.5	www.amazon.com	Other
O3	2 Pcs/Set Mini Bubble Level	1	5.66	5.66	www.amazon.com	Non-specific

Items O1 – O3 are optional.

4. Build instructions

1. Rotating stage (Fig. 2): Modify the turntable (A1) or a similar turntable for the rotating stage of the container, by removing the dust cover, the decorative ring, the rubber mat, and the tonearm from the turntable and then milling out the center shaft.

Place the cylindrical transparent container (A2) or a similar container on the turntable platter of the modified turntable (A1). Align the center of the container carefully with respect to the center of the turntable platter. Double-sided tape (O1) can be used to fix the bottom of the container to the turntable platter more securely.

2. Wheel box (Fig. 3a): Assemble two pattern mounts (B1) and the channel plate (B2) into a box. Attach eight wheels to the box by assembling wheel standoffs (B3) and wheels (B4) using button head hex screws (B5). Socket head cap screws (B0) are used for all other steps if not specified.

3. Linear stage (Fig. 3b): Insert the lead screw (B6) through the center holes of the wheel box (Fig. 3a). Insert two screw nuts (B7) to the lead screw to sandwich the wheel box (two B7 parts near the wheel box in Fig. 3b). These nuts are used to fasten the wheel box from both sides, to fix the location of the wheel box during operation. Then, slide two X-rail parts (B8) through the wheels of the wheel box while aligning the wheels with the groove of the X-rail part (Fig. 3d). Attach four brackets (B9) to the side of the X-rail parts using screw plates (B10; this screw plate is indicated by the dashed arrow in Fig. 3c because it is invisible). After that, adjust these two X-rail parts (B8) so that they are parallel with the lead screw (B6), and fix their both ends using pattern plates (B11). At the end, use two screw nuts (B7) to fix the two ends of the lead screw to the pattern plates (B11) (Fig. 3c). Attach one screw plate (B10; this screw plate is visible) to each bracket (B9) as shown in Fig. 3c. These screw plates are necessary to fix the linear stage on top of the main frame later, and the position of the brackets needs to be adjusted in that step.

4. Syringe needle holder (Fig. 4): The barrel part of the syringe (C1) is modified into the needle holder. Drill two holes on the flange of the syringe (Fig. 4a). The size and position of holes should be matched to the screw holes at the end of the X-rail part (C2) (see the inset of Fig. 4c). Insert the tubing of a desired length (C3) into the syringe barrel, and pull out the tubing out of the syringe nozzle up to a few centimeters. Then, pour prepared epoxy (C4) into the syringe barrel carefully, not to block the inside of the tubing (Fig. 4b). Once the epoxy is cured, cut the tubing end coming out of the syringe nozzle to an appropriate length.

Insert the other end of the tubing into the center hole of the one side of the X-rail part (C2), and pull it out from the other end. Fix the syringe flange onto the X-rail part (Fig. 4c inset). Attach the syringe needle (C5) to the syringe nozzle (Fig. 4c). The syringe needle can be replaced with different needles.

5. Main frame (Fig. 5): Build the base of the frame first (Fig. 5a). Cut two pairs of X-rail parts (B8 and B12) into 46.5 cm and 37 cm, respectively, so that the base can fit with the turntable (A1). Array these parts in a rectangular shape, and fix each corner of the base using one L-bracket (B13) and two screw plates (B10) (Fig. 5b).

Attach four X-rail parts (B8) vertically to the frame base using flush mounts (B14), T-brackets (B15), and screw plates (B10) as shown in Fig. 5c. If necessary, an extra T-bracket (B15) and screw plates (B10) can be added to the opposite side of the joint for more stable assembly.

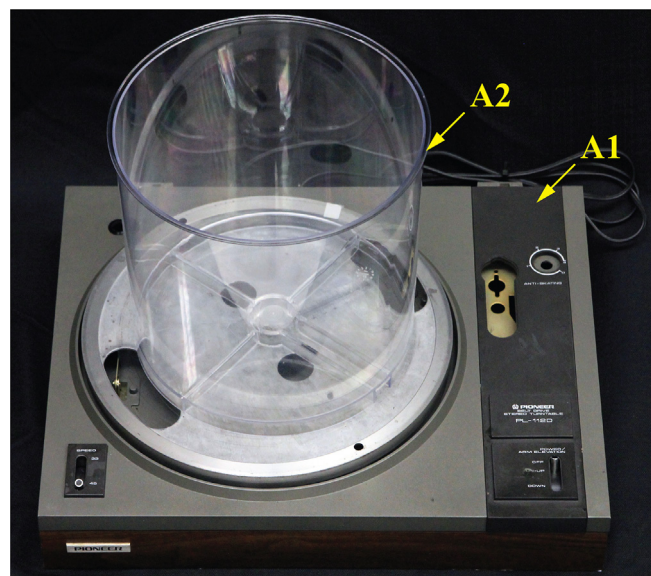


Fig. 2. The modified turntable (A1) and the cylindrical container (A2) for rotating CP and CL liquids in rigid body motion.

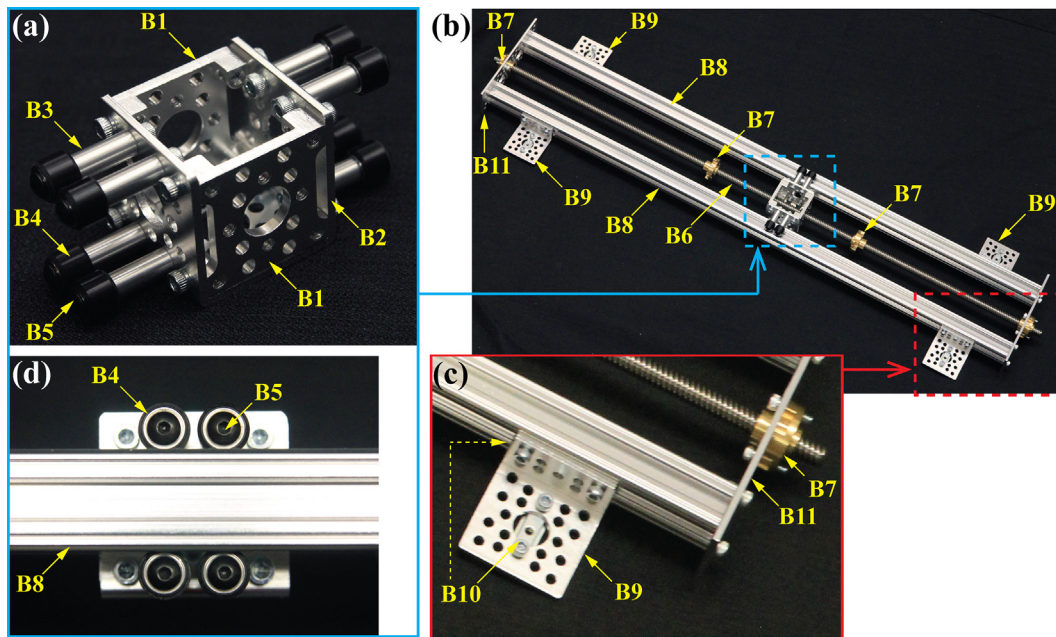


Fig. 3. Linear stage. (a) Wheel box. (b) Assembled linear stage. (c) Magnified view of the end part of the linear stage. The screw plate (B10) fixing the bracket (B9) on the X-rail part is invisible because it is covered by the bracket (indicated by the dashed arrow). (d) The X-rail part (B8) slides between the wheels (B4) of the wheel box.

In the same way, add two X-rail parts (B12) to the top of the four vertical X-rail parts (Fig. 5d) horizontally. Then, add two X-rail parts (B8) to the both ends of the horizontal X-rail parts, to form a rectangular frame. The finished main frame is shown in Fig. 5d.

6. Assembly (Fig. 6a): Place the turntable (A1) and the container (A2) at the center of the frame base. If necessary, re-center the container on the turntable. Remove one of the horizontal X-rail parts (B8) from the top of the frame (Fig. 5d). Carefully slide the screw plates (B10) attached to the bracket (B9) of the linear stage (Fig. 3c) into the groove of the top X-rail parts (B12) of the frame so that the linear stage can sit on the main frame. If necessary, adjust the position of the brackets (B9). Reassemble the detached X-rail part (B8) to the top of the frame.

Adjust the linear stage so that its lead screw (B6) can be aligned with the center of the container, and fix the stage firmly on the frame. Pass the free end of the tubing of the syringe holder (C3) through the center of the wheel box, and fix the needle holder to the bottom of the wheel box. Then, connect the free end of the tubing to a syringe pump (Chemyx Fusion 200 was used in this paper) or any available fluid injection device. Move the wheel box to the center of the container, and mark the position of the wheel box on the X-rail part (B8) of the linear stage as a reference point. The distance between the axis of rotation of the container and the needle can be determined by measuring the distance from the reference point to the wheel box after the needle holder is moved. An optional ruler tape (O2) can be attached to the X-rail part (B8) of the linear stage as a reference for the needle position. In addition, optional water levels (O3) can be used to check whether the needle holder is vertical and whether the turntable is horizontal.

5. Operation instructions

1. Choose a pair of CP, DP and CL liquids, and determine desired operating conditions such as the size of the needle (D_i), the injection volume flow rate of the DP liquid (Q), the rotating speed of the turntable (ω), and the distance from the syringe needle to the axis of rotation of the container (R).
2. Fill the container (A2) with the CP and CL liquids. Let them stand to form two immiscible liquid layers.
3. Connect the tubing (C3) to the syringe pump for DP liquid injection.

(Optional) The syringe pump can be replaced by any fluid injection device. There are two options for injecting the DP liquid if a commercial syringe pump is not available. One is to use gravity to drive the DP flow. Place a container of the DP liquid at a higher location than the needle, and submerge the free end of the tubing in the DP liquid in this container. Then, the DP liquid can be injected into the rotating CP liquid due to hydrostatic pressure. The other is to fabricate an open source syringe pump for more accurate controls [8,9].

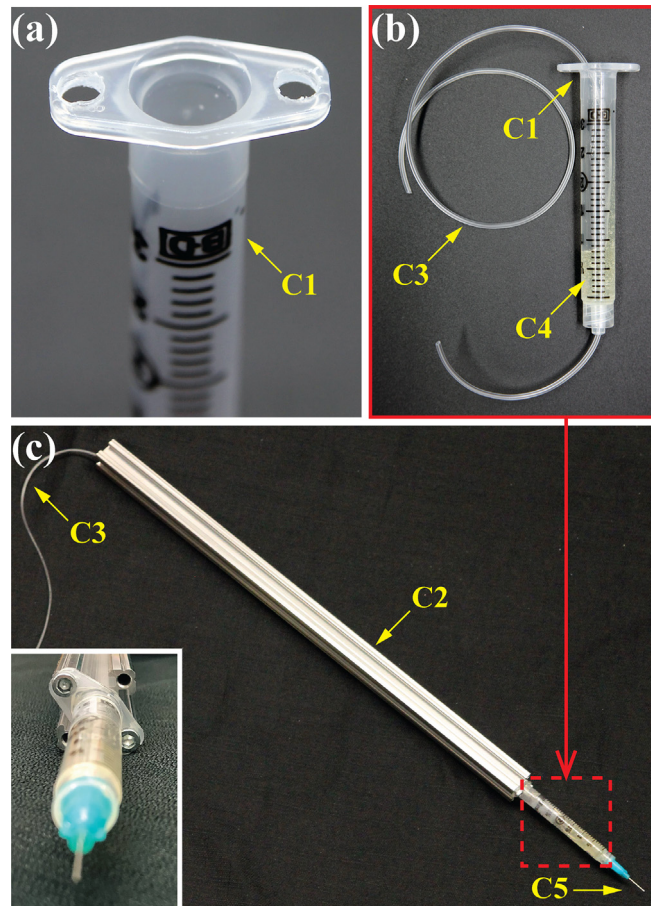


Fig. 4. Syringe needle holder. (a) Two holes are drilled on the flange of the syringe (C1). (b) The syringe (C1) with tubing (C3) fixed with epoxy (C4). (c) Assembled syringe needle holder with a replaceable syringe needle (C5). The inset shows how the modified syringe is attached to the X-rail part (C2).

(Optional) The needle (C5) can be replaced by different syringe needles.

4. Move the needle holder to a desired location, and fix it by fastening the wheel box using the two screw nuts (B7) to sandwich the wheel box.
5. Set the rotation speed of the turntable (A1).
6. Set the volume flow rate of the syringe pump.
7. Turn on the turntable (A1) and wait until the free surface of the CP liquid reaches the steady parabolic shape.
8. Turn on the syringe pump to start injecting the DP liquid. Drop generation will start immediately after air is expelled from the tubing, and drops will be generated continuously at the needle tip (C5).
9. Wait till enough number of DP drops reach the interface between the CP and CL liquid and become hydrogel beads in the CL liquid, and then turn off the syringe pump and the turntable (A1).
10. Collect hydrogel beads from the bottom of the container (A2).

6. Validation and characterization

6.1. Drop generation

The drop generation capability of the proposed rotating-liquid-based hydrogel bead generator was investigated under several different conditions [10]. In this test, deionized water (DI water; DP liquid) was injected into rotating mineral oil (05089-cr, Bluewater Chemgroup; CP liquid) through the syringe needle with the inner diameter of $D_i = 0.32$ mm. The rotation speed of the turntable was $\omega = 33.7$ and 42.7 revolutions per minute (rpm), which equal to 3.5 and 4.5 rad/s, respectively. The horizontal distance from the axis of rotation of the turntable to the needle was $R = 80$ mm. Therefore, the linear speed of the oil motion at the needle tip ($V_c = \omega R$) was 0.28 and 0.36 m/s. DI water was injected by the syringe pump at volume flow rates of $Q = 1, 2, 4,$ and 6 mL/min. Thus, the average speed of DI water flow at the needle tip [$V_d = 4Q/(\pi D_i^2)$]

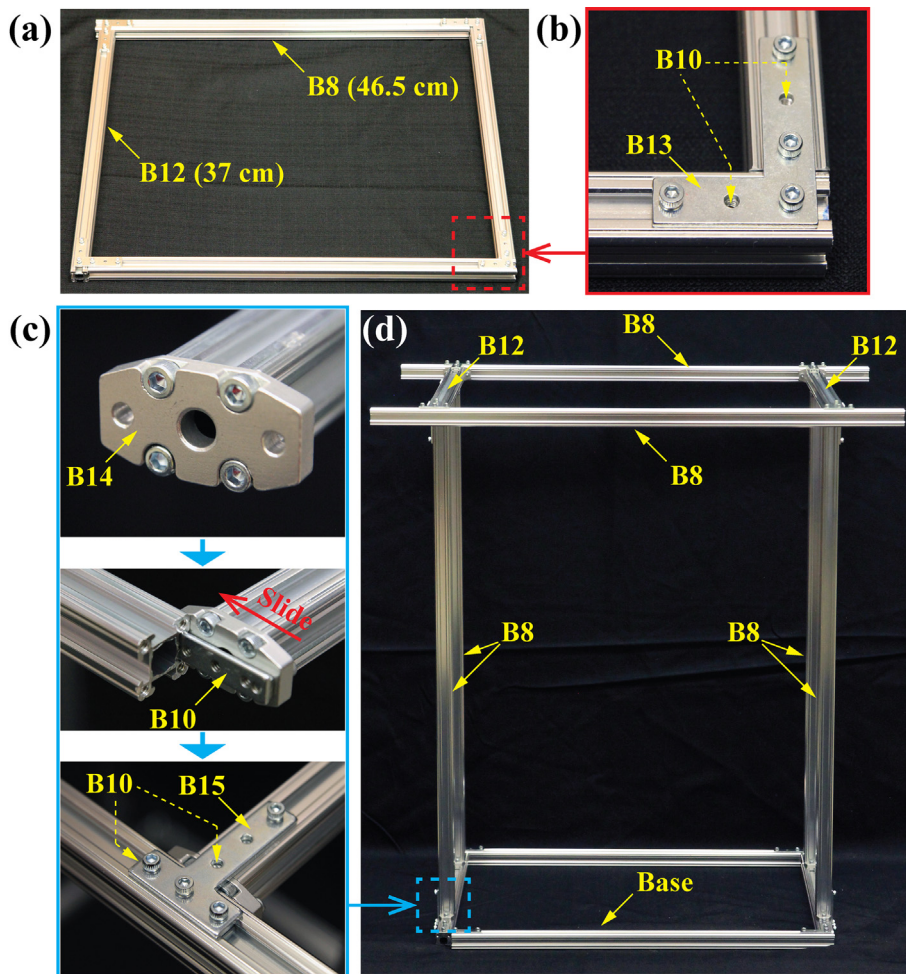


Fig. 5. Main frame. (a) The base of the frame. (b) Magnified view of the assembled corner of the base. Two screw plates (B10) are under the L-bracket (B13). (c) Detailed procedure of assembling two X-rail parts perpendicularly. Top: Attach one flush mount (B14) to one end of an X-rail part. Middle: Attach one screw plate (B10) to the pattern mount, and slide it through the groove of another X-rail part. Bottom: Fix the joint using one T-bracket (B15) and two screw plates (B10). The screw plates are inserted in the grooves of the X-rail parts and under the T-bracket. (d) Assembled main frame.

was 0.21, 0.41, 0.83, and 1.24 m/s. All combinations of V_c and V_d were tested to generate drops of different sizes as validation, and drops being produced were captured by a high-speed camera (Phantom MIRO M310, Vision Research) with a macro lens (EF 100 mm f/2.8 macro lens, Canon). Fig. 6b shows an example of drop generation with operating conditions of $R = 40$ mm, $\omega = 3.5$ rad/s, and $Q = 0.5$ mL/min (thus, $V_c = 0.14$ m/s, and $V_d = 0.10$ m/s) (see Movie S1).

As the left image of Fig. 7a shows, captured images showed elliptical drops. Although actual drops were spherical due to the surface tension between the DP and CP liquids, they looked stretched in the horizontal direction due to the curved wall of the cylindrical container. Thus, captured images were corrected for the image distortion based on calibration using a grid plate made as follows. A grid of 5 mm \times 5 mm squares was printed on a transparency film, and the film was cut and glued onto a slide glass. A reference image of the grid plate for correction was captured when the grid plate was attached vertically to the needle holder instead of the needle (C5) for each distance of R . As the left image of Fig. 7b shows, the square grid was stretched and appeared rectangular on the captured image. For image correction, the coordinates of the intersections of the grid pattern were digitized using ImageJ [11], and the tilting angle and aspect ratio of the squares were calculated. Image correction using these values has removed distortion from the grid image, and thus drops of DI water look spherical in the corrected images (the right images of Fig. 7).

After image correction, drop images were post-processed by using Droplet Morphometry and Velocimetry (DMV) [12] to measure the diameter of produced drops (D_{drop}). Fig. 8 summarizes the results of drop diameters in the format of $m \pm \sigma$ [CV], where m and σ are the mean and standard deviation of D_{drop} , and CV is the coefficient of variation of D_{drop} ($CV = \sigma/m$). The results show that the rotating-liquid-based hydrogel bead generator can generate drops of different diameters by modulating the operation parameters, and thus hydrogel beads of various diameters can be produced.

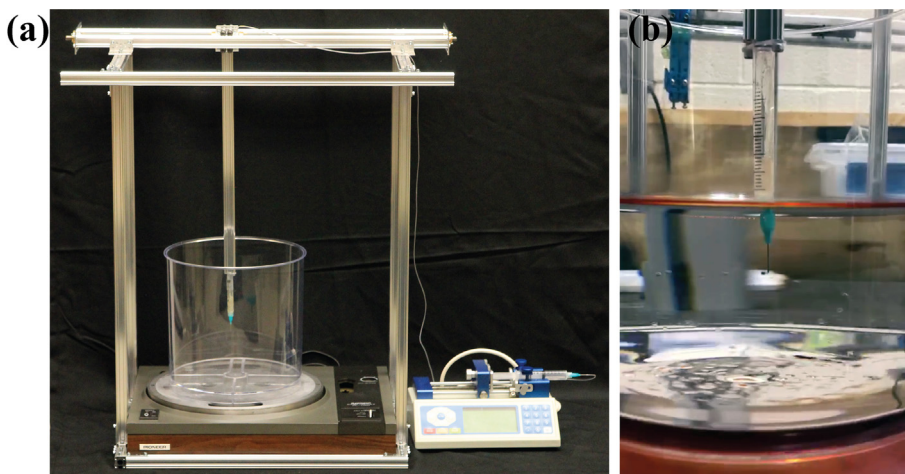


Fig. 6. Rotating-liquid-based hydrogel bead generator. (a) Finished setup. The needle (C5) is placed at the center of the container (A2), and the syringe needle is connected to a syringe pump. (b) An example of drop generation with operating conditions of $R = 40$ mm, $\omega = 3.5$ rad/s, and $Q = 0.5$ mL/min (thus, $V_c = 0.14$ m/s, and $V_d = 0.10$ m/s) (See [Movie S1](#)).

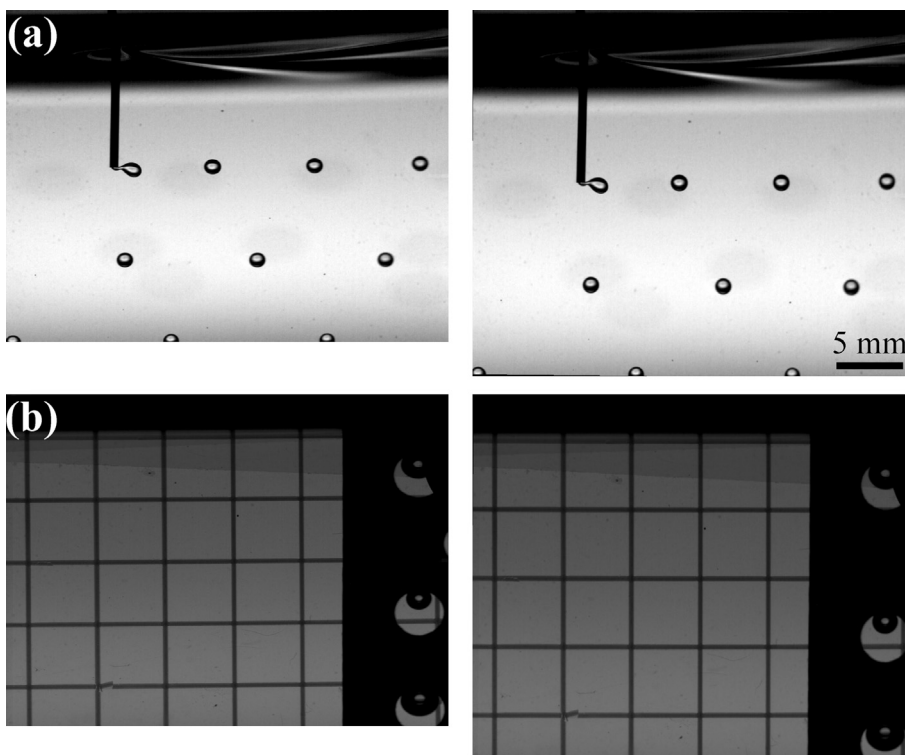


Fig. 7. Correction of image distortion due to the cylindrical wall of the container. (a) Deformed (left) and corrected (right) drop images. (b) Deformed (left) and corrected (right) image of the grid plate.

Also, drops were observed to be generated in either the dripping or jetting mode [10,13,14]. In the dripping mode, drops were pinched off at or near the needle tip (cases of $V_d = 0.21$, 0.41 , and 0.83 m/s in [Fig. 8](#); see [Movie S2](#) [$V_c = 0.28$ m/s and $V_d = 0.41$ m/s]) whereas in the jetting mode, the drops were pinched off from the DP stream jetting from needle tip (case of $V_d = 1.24$ m/s in [Fig. 8](#); see [Movie S3](#) [$V_c = 0.28$ m/s and $V_d = 1.24$ m/s]). The distance from the needle tip to the pinch-off site (L) was used as a criterion to determine the jetting mode: $L > D_{drop}$ [14]. The monodispersity of produced drops was found to be better in the dripping mode as shown by the lower CV values. Therefore, it is recommended to operate the gel bead generator in the dripping mode to obtain more uniformly sized hydrogel beads.

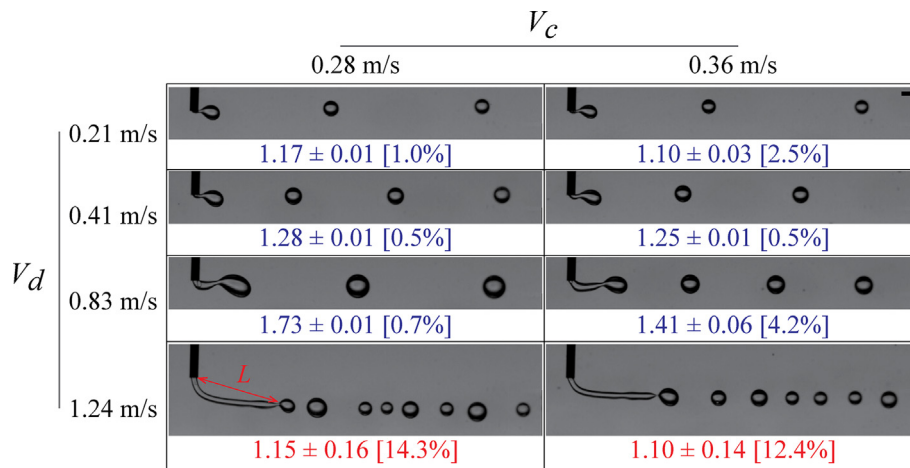


Fig. 8. Summary of drop generation results (scale bar: 1 mm). Drop diameter in each case is shown in the format of mean value (m) \pm standard deviation (σ) (unit: mm). Coefficient of variation ($CV = m/\sigma$) is shown in brackets. V_c and V_d are the speed of the CP liquid (mineral oil) at the needle tip and the average speed of the DP liquid (DI water), respectively. Blue and red color indicate the dripping and jetting mode, respectively.

It is ideal for each user of the method to determine D_{drop} experimentally, but our approach based on high speed imaging cannot be easily adopted. Instead, we suggested empirical, power-law relations between averaged D_{drop} (m) and V_d ($m = \alpha V_d^\beta$) or V_c ($m = \alpha V_c^\beta$) as a reference for predicting the size of drops produced in the dripping mode [10]. The values of coefficient α and β were determined by fitting the power-law equation against experimentally measured D_{drop} data. For the syringe needle (C5) used in this study, α and β in $m = \alpha V_d^\beta$ ranged from 1.40 to 2.47, and from 0.07 to 0.10, respectively, for a range of $0.14 \text{ m/s} \leq V_c \leq 0.38 \text{ m/s}$, and α and β in $m = \alpha V_c^\beta$ ranged from 0.46 to 0.66, and from -0.71 to -0.63 , respectively, for the range of $0.02 \text{ m/s} \leq V_d \leq 0.41 \text{ m/s}$. For further details of the suggested empirical relations, refer to Ref. [10].

6.2. Alginate gel bead generation

The hydrogel bead generation of the proposed method was demonstrated using alginate as follows. PEG-10 dimethicone (KF-6017, Shin-Etsu Chemical) was added to mineral oil at a concentration of 0.2 g/L as a surfactant. Then, 2.4 L of the mineral oil and 0.2 L of 10% w/v calcium chloride solution (C4901, Sigma-Aldrich) were poured into the container (A2) as the CP and CL liquids, respectively [15]. After resting, the CP and CL liquids were separated into the top and bottom layers, respectively. Alginate solution was prepared as the DP fluid by dissolving 1.5% w/v alginate gel powder (A0682, Sigma) and 15.7% w/v sodium chloride (S5886, Sigma) in $1 \times$ phosphate buffered saline (PBS; K813, Amresco) in a hot water bath [16]. Water can be used instead of PBS. The Young's modulus of alginate gel of this concentration is about 40 kPa [17].

The drop generation conditions were set as $Q = 0.5 \text{ mL/min}$, $\omega = 3.5 \text{ rad/s}$, and $R = 80 \text{ mm}$, and the tip of the syringe needle (C5) was located 10 mm below the free surface of the CP liquid. Thus, V_c and V_d were 0.28 m/s and 0.11 m/s, respectively. After the turntable (A1) was turned on, the DP liquid was injected through the needle (C5), and drops of the alginate solution were generated continuously. Because of the density difference between the CP and DP liquids, DP drops fell down while rotating with the rigid body motion of the CP liquid. Once DP drops reached the interface between the CP and CL liquids, the gelation of DP drops were triggered immediately by the calcium ion in the CL liquid. Afterwards, the alginate gel beads settled down through the CL liquid. When enough number of alginate gel beads were generated, the setup was turned off, and then the gel beads were harvested manually by transferring them to another container by a pipette or spoon.

As shown in Fig. 9, produced alginate gel beads showed similar size, and they were in the tear drop shape rather than the spherical shape. Although the surfactant in the CP liquid appeared to help DP drops to maintain the spherical shape when they made a contact with the CP-CL interface [18], the shape of the drop seems to have changed during gelation. Upon the contact, the gelation process began at the lower part of the drop first. As the drop entered the CL layer, the gelation in the upper part seemed to follow. During this asymmetric gelation, the drop seemed to be shaped into the tear drop shape. Thus, the observed tear drop shape appears to be due to the slow pass of the alginate drops through the CP-CL interface and the time lag in gelation between the lower and upper part of the drops [19]. Similar tear-drop-shaped alginate beads were reported in previous studies using external ion cross-linking and microfluidics [19–21]. Because the gel beads were not spherical, their equivalent diameters (i.e., the diameter of a circle with the same area as the bead) were measured using DMV [12]. The mean and standard deviation of the bead diameter were 0.66 mm and 0.08 mm ($N = 227$ beads), respectively. The presented demonstration results clearly show that the rotating-liquid-based hydrogel bead generator can successfully produce alginate gel beads of uniform size and shape.

The proposed method can be used for fabricating different types of hydrogel beads. For example, polyacrylamide hydrogel beads can be made by injecting its pre-polymer solution, which is a mixed solution of acrylamide, bis-acrylamide, and a

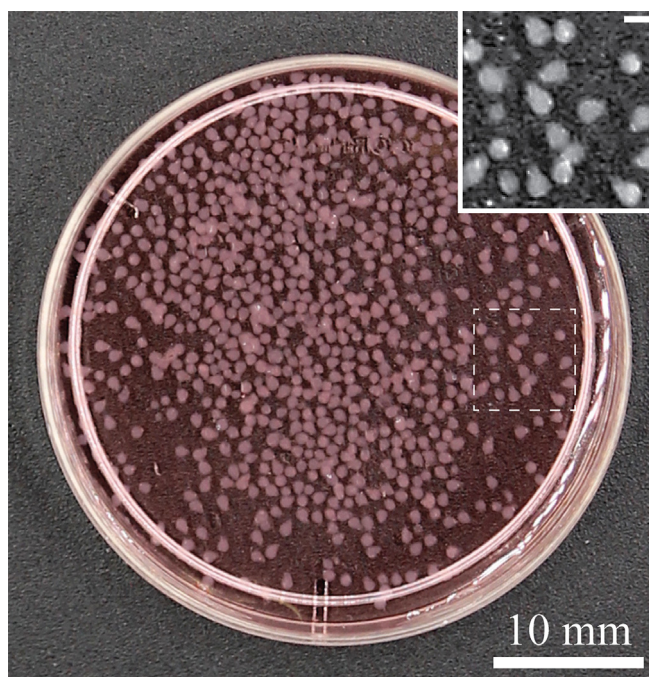


Fig. 9. Alginate hydrogel beads produced by the rotating-liquid-based hydrogel bead generator ($V_c = 0.28$ m/s and $V_d = 0.11$ m/s). A small amount of red dye was added to water to enhance the visibility of the gel beads. Inset: Magnified view of the white dashed box (scale bar: 1 mm).

photo-initiator such as Irgacure 2959, as the DP liquid in rotating mineral oil, and then exposing created DP drops to UV light to trigger cross-linking [22]. In this case, the CL liquid is not required, and the requirement for gelation time and UV light (e.g., 15 min with wavelength of 365 nm [23]) needs to be considered in modifying the proposed gel bead generator. For instance, the CP layer needs to be thick enough for falling DP drops to be exposed to UV light long enough and thus to complete their gelation before arriving at the bottom of the container. Also, UV light irradiation needs to be carefully incorporated in the setup to avoid any undesirable effects of the container wall, such as refraction and reflection of UV light. The simplicity and versatility of the proposed method enable modifying the setup easily for a wide range of hydrogel materials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ohx.2020.e00121>.

References

- [1] P. Zhu, L. Wang, Passive and active droplet generation with microfluidics: A review, *Lab Chip* 17 (2017) 34–75.
- [2] G.T. Vladislavjević, I. Kobayashi, M. Nakajima, Production of uniform droplets using membrane, microchannel and microfluidic emulsification devices, *Microfluid. Nanofluid.* 13 (2012) 151–178.
- [3] S.Y. Teh et al, Droplet microfluidics, *Lab Chip* 8 (2008) 198–220.
- [4] R. Seemann et al, Droplet based microfluidics, *Rep. Prog. Phys.* 75 (2011) 16601.
- [5] A.M. Pit, M.H.G. Duits, F. Mugele, Droplet manipulations in two phase flow microfluidics, *Micromachines* 6 (2015) 1768–1793.
- [6] D. Dendukuri, P.S. Doyle, The synthesis and assembly of polymeric microparticles using microfluidics, *Adv. Mater.* 21 (2009) 4071–4086.
- [7] B.G. Chung et al, Microfluidic fabrication of microengineered hydrogels and their application in tissue engineering, *Lab Chip* 12 (2012) 45–59.
- [8] V.E. Garcia, J. Liu, J.L. DeRisi, Low-cost touchscreen driven programmable dual syringe pump for life science applications, *HardwareX* 4 (2018) e00027.
- [9] K. Pusch, T.J. Hinton, A.W. Feinberg, Large volume syringe pump extruder for desktop 3D printers, *HardwareX* 3 (2018) 49–61.
- [10] H. Zhang, S. Ryu, Drop generation in cross-flow of liquid rotating in rigid body motion, *J. Fluid Eng.* 142 (2020) 104501.

- [11] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH image to ImageJ: 25 years of image analysis, *Nat. Methods* 9 (2012) 671.
- [12] A.S. Basu, Droplet Morphometry and Velocimetry (DMV): A video processing software for time-resolved, label-free tracking of droplet parameters, *Lab Chip* 13 (2013) 1892–1901.
- [13] R.F. Meyer, J.C. Crocker, Universal dripping and jetting in a transverse shear Flow, *Phys. Rev. Lett.* 102 (2009) 194501.
- [14] A. Bertrandias et al, Dripping to jetting transition for cross-flowing liquids, *Phys. Fluids* 29 (2017) 44102.
- [15] K. Aketagawa, H. Hiram, T. Torii, Hyper-miniaturisation of monodisperse Janus hydrogel beads with magnetic anisotropy based on coagulation of Fe₃O₄ nanoparticles, *J. Mater. Sci. Chem. Eng.* 1 (2013) 34828.
- [16] D. Lee et al, Pneumatic microfluidic cell compression device for high-throughput study of chondrocyte mechanobiology, *Lab on a Chip* 18 (2018) 2077–2086.
- [17] D. Lee, H. Zhang, S. Ryu, Elastic modulus measurement of hydrogels, in *Cellulose-based Superabsorbent Hydrogels*, M.I.H. Mondal, Editor. 2019, Springer International Publishing, p. 865–884. https://link.springer.com/referenceworkentry/10.1007%2F978-3-319-76573-0_60-1.
- [18] M. Schmitt et al, Effect of SPAN80 on the structure of emulsified aqueous suspensions, *Col. Surf. A* 521 (2017) 121–132.
- [19] L. Capretto et al, Effect of the gelation process on the production of alginate microbeads by microfluidic chip technology, *Lab Chip* 8 (2008) 617–621.
- [20] Y. Hu et al, Shape controllable microgel particles prepared by microfluidic combining external ionic crosslinking, *Biomicrofluidics* 6 (2012) 26502.
- [21] M.D. Eqbal, V. Gundabala, Controlled fabrication of multi-core alginate microcapsules, *J. Col. Interf. Sci.* 507 (2017) 27–34.
- [22] D. Lee et al, Fabrication of hydrogels with a stiffness gradient using limited mixing in the Hele-Shaw geometry, *Exp. Mech.* 59 (2019) 1249–1259.
- [23] N.F. Ayub et al, New UV LED curing approach for polyacrylamide and poly(N-isopropylacrylamide) hydrogels, *New J. Chem.* 41 (2017) 5613–5619.