Lab on a Chip

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# Human stomach-on-a-chip with luminal flow and peristaltic-like motility

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## ABSTRACT

Current *in vitro* approaches and animal models have critical limitations for modeling human gastrointestinal diseases because they may not properly represent multicellular human primary tissues. Therefore, there is a need for model platforms that recapitulate human *in vivo* development, physiology, and disease processes to validate new therapeutics. One of the major steps toward this goal was the generation of three-dimensional (3D) human gastric organoids (hGOs) via the directed differentiation of human pluripotent stem cells (hPSCs). The normal functions and diseases of the stomach occur in the luminal epithelium, however accessing the epithelium on the inside of organoids is challenging. We sought to develop a bioengineered platform to introduce luminal flow through hGOs to better model *in vivo* gastric functions. Here, we report an innovative microfluidic imaging platform housing hGOs with peristaltic luminal flow *in vitro.* This human stomach-on-a-chip allows robust, long-term, 3D growth of hGOs with the capacity for luminal delivery via a peristaltic pump. Organoids were cannulated and media containing fluorescent dextran was delivered through the lumen using a peristaltic pump. This system also allowed us to rhythmically introduce stretch and contraction to the organoid, reminiscent of gastric motility. Our platform has the potential for long-term delivery of nutrients or pharmacological agents into the gastric lumen *in vitro* for the study of human gastric physiology, disease modeling, and drug screening, among other possibilities.

## 1. Introduction

Conventional drug development is a time-consuming procedure accompanied by significant cost and low efficiency [1]. This is mainly attributed to the poor predictive power of physiologically relevant preclinical models for establishing drug efficacy and safety, leading to the low success rates during clinical trials [2]. Cancer cell lines and rodent models do not fully recapitulate human physiology or pathology. Moreover, animal models are low throughput and carry an ethical burden [3]. Thus, these limitations highlight the critical need for high throughput screening tools that recapitulate human *in vivo* microenvironments to discover and validate new therapeutics.

Toward this end, a number of studies have recently reported *in vitro* generation of three-dimensional (3D) human organoids, which recapitulate the development, structure and microenvironment of human organs [4-8]. We previously developed a robust protocol for generating human gastric organoids (hGOs) via the directed differentiation of human pluripotent stem cells (hPSCs) [9,10]. hGOs recapitulate the tissue architecture of human stomach and represent a new, powerful tool for unprecedented *in vitro* studies of human development, genetic and infectious disease, and cancer [7-9,11]. However, there are critical barriers that hinder major advancements for studies utilizing the gastric organoid model: Paucity of high-throughput experimental manipulation in traditional tissue culture formats, and absence of physiological luminal flow through organoids.

Another *in vitro* model that recently emerged is the human "microphysiological" or "organ-on-a-chip" platform, in which significant advances in microscale technologies allow the recapitulation of the physiological microenvironments for human tissues and organs [2,3,12]. These organ-like platforms enable more accurate prediction of human physiology, and more

importantly, they provide the potential to generate specialized *in vitro* human disease models that could advance drug development [2,3,12-16]. A human gut-on-a-chip platform has been developed to mimic the normal *in vivo* intestinal microenvironment [13,17,20]. This platform was composed of two perfusable microchambers (upper and lower) separated by a porous polydimethylsiloxane (PDMS) membrane (> 10 μm thickness), and was used to evaluate absorption of orally administrated drugs. The flexible PDMS membrane was coated with extracellular matrix (ECM) and lined by human Caco2 intestinal epithelial cells. The gut microenvironment was remodeled by flowing fluid at a low rate (30 μL/hr) producing low shear stress over the microchambers. Cyclic suction was applied to both microchambers to mimic the mechanically active microenvironment of small intestine [17]. Although this model was able to recapitulate parts of intestinal physiology and pathology, there are still critical limitations and challenges. First of all, most microphysiological devices are made from flexible clear polymer PDMS, as it allows real-time optical imaging of cells due to its transparency, and is cheap and easy to work with in the laboratory. However, it also absorbs small hydrophobic molecules, including certain drugs, fluorescent dyes, or cell signaling molecules. This potentially might cause reduction of effective drug concentrations, cross-contamination, and lower detection sensitivities, leading to unreliable results. Furthermore, when the non-degradable PDMS membrane was used as a support for epithelium in the human gut-on-a-chip, it was technically challenging to fabricate a biologically relevant thickness. The membrane used in the human gut-on-a-chip design [17, 20] was commonly over 10 μm in thickness, whereas the physiological basement membrane is only about 400 nm thick. This huge difference in thickness (> 10 μm vs. 400 nm) may significantly impede drug transport kinetics between intestine and blood stream as well as prevent integration of other tissue types into the system, such as macrophages, blood vessels, or neurons. Finally, monolayer culture

of intestinal epithelial cells may be insufficient to recapitulate the complex, 3D morphology of gastrointestinal organs. Up to now, there has been no experimentally feasible *in vitro* platform of producing luminal flow with gastric motor activity.

In this study, we created an innovative imaging platform housing hGOs with steady-state luminal flow, leading to a peristaltic human stomach-on-a-chip *in vitro* (Fig. 1). We demonstrate that our platform allows for robust 3D growth of gastric organoids with the capabilities for long-term time-course optical imaging. Furthermore, our *in vitro* system generates a microphysiological platform that mimics *in vivo* luminal flow, which is instrumental in studying gastric diseases and provides a new platform for screening oral drugs *in vitro*.

## 2. Materials and methods

### *2.1. Device fabrication*

Fig. 2 presents the fabrication procedure for the human stomach-on-a-chip imaging platform. Schematic 3D structures for the mold and imaging platform were designed and modeled using SolidWorks 2017 (Dassault Systems SolidWorks Corp) CAD package as shown in Fig. 2A and 2B. To fabricate the human stomach-on-a-chip imaging platform, a polymer master mold was successfully printed in a 3D printing system (Form 2, Formlabs, Inc.). The material for the 3D printing was Tough V4 polymer that is a photopolymer resin from Formlabs, Inc. The 3D stomach-on-a-chip imaging platform was built with PDMS (10:1 w/w; Sylgard 184, Dow Corning) by a standard PDMS replica molding technique [20]. The surface of the cover glasses was cleaned using 1:5 ratio of (HCl:H2O) (v/v) solution for 10 min, and the isopropanol was used to clean the surface of PDMS replica. After the cleaning process, both surfaces were activated using oxygen plasma treatment (CS-1701 RIE, MARCH Instruments) to generate surface hydroxyl groups.

Finally, the stomach-on-a-chip was fabricated by spontaneous bonding between the patterned PDMS replica and cover glass (Fig. 2C). Experimental setup for long-term luminal flow of stomach-on-a-chip was described in Fig. 2D, with a central chamber for culturing the organoid and two in-line chambers for media.

*2.2. Generation of human gastric organoids*

hGOs were generated as previously described [8,9,17,18]. Briefly, hPSCs were maintained on hESC-qualified Matrigel (BD Biosciences) in mTesR1 medium without feeders in a standard tissue culture incubator set to 37ºC and 5% $CO_2$. Prior to initiating the differentiation protocol, hPSCs were dissociated into single cells using Accutase (Thermo Fisher Scientific) and passaged into a 24-well plate in mTeSR1 supplemented with Y-27632 (10 uM; Tocris). The following day, cells were treated with Activin A (100 ng/mL, R&D Systems) for three consecutive days in RPMI 1640 media (Invitrogen) supplemented with NEAA (1x; Gibco) and increasing concentrations of 0%, 0.2%, 2% HyClone defined FBS (dFBS) (Thermo Scientific) to induce differentiation into definitive endoderm. Additionally, BMP4 (50 ng/mL; R&D System) was added on the first day of Activin A treatment. After definitive endoderm formation, cells were cultured in RPMI 1640 media supplemented with NEAA and 2.0% dFBS for three days with the indicated combinations of growth factors and/or chemical agonist: CHIR99021 (2 μM; Stemgent); FGF4 (500 ng/mL; R&D Systems); and Noggin (200 ng/mL; R&D Systems). Retinoic acid (RA) (2 μM; Sigma Aldrich) was added on the final day, with the media being changed every day. After three days, the combination of CHIR99021, FGF4, Noggin, and final day RA resulted in floating posterior foregut spheroids in the culture wells. Spheroids were collected and embedded in 50 μL Matrigel (Corning), and plated as 3D Matrigel droplets with ~100 spheroids per droplet. After

the Matrigel droplets were allowed to solidify for 10–15 min in a tissue culture incubator, spheroids were overlaid with gut media: Advanced DMEM/F12 (Gibco) supplemented with: N2 (1x; Invitrogen), B27 (without Vitamin A) (1x; Invitrogen), 2mM L-glutamine (1x; Life Technologies), HEPES (15mM; Gibco), 100 units/mL penicillin/streptomycin (1x; Life Technologies), and EGF (100 ng/mL; R&D Systems). For the first 3 days after Matrigel embedding, RA and Noggin were added to the gut media. Media was replaced every 3–4 days, as necessary. Approximately two weeks after Matrigel embedding, organoids were collected and re-embedded at a reduced density in fresh Matrigel with ~ 3 organoids per Matrigel droplet to allow for additional unconfined growth.

*2.3. Short-term culture of single gastric organoid on a platform*

Approximately two weeks after reducing density, a single antral hGO was collected and re-embedded into the central chamber of a stomach-on-a-chip platform with fresh Matrigel. The Matrigel droplet was allowed to solidify for 10-15 min in a tissue culture incubator before media (450 μL each) was inserted to the two in-line chambers. The platform immediately returned to the tissue culture incubator and was allowed to equilibrate for approximately 3 hours.

*2.4. Establishment of and imaging luminal flow in a single gastric organoid*

After the organoid had equilibrated within the platform, the hGO-on-a-chip platform was securely transferred to a microscopy system for establishment of luminal flow through the hGO. Two borosilicate micropipettes (~50 μm in tip diameter) were inserted into opposing sides of the hGO, and the micropipettes were directly connected to tubing (1/16 inch outside diameter, IDEX Health & Science LLC) run through a peristaltic pump for biomimetic periodic pumping (Instech

Laboratories Inc.). Prior to puncturing the hGO, the "in-flow" needle and tubing were primed and backfilled with fluorescein isothiocyanate (FITC) dextran solution (3 kDa molecular weight) diluted in phosphate-buffered saline (Sigma Chemical Co.). We integrated the platform, two micropipettes, tubing and the peristaltic pump on the stage of a Nikon AZ100 upright microscope with an Andor Zyla 4.2 PLUS sCMOS camera to acquire live bright-field and fluorescent images. Brightfield images were taken using reflected light provided by a fiber optic source. Fluorescence images of FITC dextran were taken utilizing a Lumencor SpectraX light source providing illumination at 470nm, a 495nm dichroic mirror and a 525/50 bandpass filter. Fluorescence images were taken with the Andor Zyla 4.2 PLUS camera in 16bit mode. Images were acquired in two separate setups, which were at an interval of one second for Fig. 3 and 1 frame every four seconds for Fig. 4.

### *2.5. Image processing and quantitative analysis*

Images were processed and quantitatively analyzed with Nikon Elements 5.02 software. We used this software to acquire data, analyze images, and to create fluorescence movies and figure images. To better visualize intensity changes of the FITC dextran during luminal flow, images were pseudocolored using rainbow look up tables (LUTs) whereby lower intensity values are shown as blue and high intensities are shown as red. Measurements of intensity changes over time were made by creating a region of interest (ROI) adjacent to a hGO at its minimum size.

## 3. Results and discussion

We developed a simple and practical platform for generating a human stomach-on-a-chip *in vitro*. We used SolidWorks software (Fig. 2A) to design the platform and produced the master

mold (Fig. 2B) via rapid prototyping technique, additive manufacturing or 3D printing, followed by PDMS molding. This approach reduces the overall cost and time required for fabrication. The fabricated PDMS-based human stomach-on-a-chip platform (Fig. 2C) is biocompatible and permeable to oxygen. Furthermore, our developed platform does not require expensive cleanroom facilities. We embedded a single hGO in Matrigel into the central chamber of each platform and used the top and bottom chambers to guide insertion of borosilicate micropipettes into opposite sides of the hGO (Fig. 2D). The borosilicate micropipettes were backfilled with FITC-dextran and connected via flexible tubing to a peristaltic pump for generating and visualizing luminal flow through the hGO. Then, we transferred the human stomach-on-a-chip platform to a microscope for optical imaging.

We observed peristaltic movements of the FITC-dextran through the hGO (Fig. 3 and Supplementary Mov. 1). Each period of cyclic expansion and contraction directly corresponded to the speed of the peristaltic pump. Using Nikon Elements software, we pseudocolored the FITC fluorescence intensity using rainbow look-up tables (LUTs) to visualize subtle changes in fluorescent intensity over time. We observed smooth and steady flow through the hGO with rhythmic expansion and contraction, and we introduced small air bubbles to the tubing to visualize luminal churning motion. We achieved steady-state luminal flow over a sustained period of time (>30 minutes), thus demonstrating proof-of-concept for long-term delivery and observation of luminal agents. This recapitulates *in vivo* luminal flow through the stomach, and provides a novel platform for studying gastric physiology, disease, and drug discovery. Moreover, this platform allows for personalized drug screening with hGOs generated from patient-derived induced pluripotent stem cells (iPSCs).

For detailed analyses of temporal dynamics of luminal flow within the hGOs, we quantified luminal delivery of the FITC-dextran through the hGO using Nikon Elements software. We used a peristaltic pump with three rollers to engage the tubing providing the FITC-dextran to the lumen of the hGO. Each roller of the pump caused a slight pressure fluctuation of the fluid within the tubing during rotation, which resulted in change of luminal volume of the hGO. We marked an arbitrary region of interest (ROI) placed adjacent to the hGO onto the stomach-on-a-chip platform using Nikon Elements 5.02 (Fig. 4B and Supplementary Mov. 2), and measured the fluorescence intensity of this ROI over time. We observed clear periodicity in the fluorescence intensity that corresponded to the mechanics of the three rollers of the peristaltic pump engaging and disengaging. The peaks of highest fluorescence intensity occurred at regular 34-second intervals and equaled one full rotation of the pump. This periodicity was stable over time (Fig. 4C and Supplementary Mov. 3), demonstrating the potential capabilities of this platform for long-term culturing of hGOs with luminal delivery of nutrients or pharmacological agents. This experimental setup is appropriate for long-term culture experiments ranging from several days to weeks, because our peristaltic stomach-on-a-chip platform can be mounted onto a plastic plate (150 mm wide × 300 mm long × 10 mm high) with dimensions that fit into a conventional $CO_2$ incubator.

## 4. Conclusions

In conclusion, we successfully demonstrated an innovative, low-cost, microfluidic imaging platform integrated with hGOs, representing a peristaltic human stomach-on-a-chip *in vitro*. Our platform allows robust growth of 3D human gastric organoids with controllable luminal flow. Importantly, this *in vitro* model platform modeling human stomach represents a new system of drug screening to help predict the efficacy of drugs as well as improve success rate of disease

treatments using patient-derived hGOs by designing patient-specific treatments while avoiding unnecessary animal testing.

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## Figures

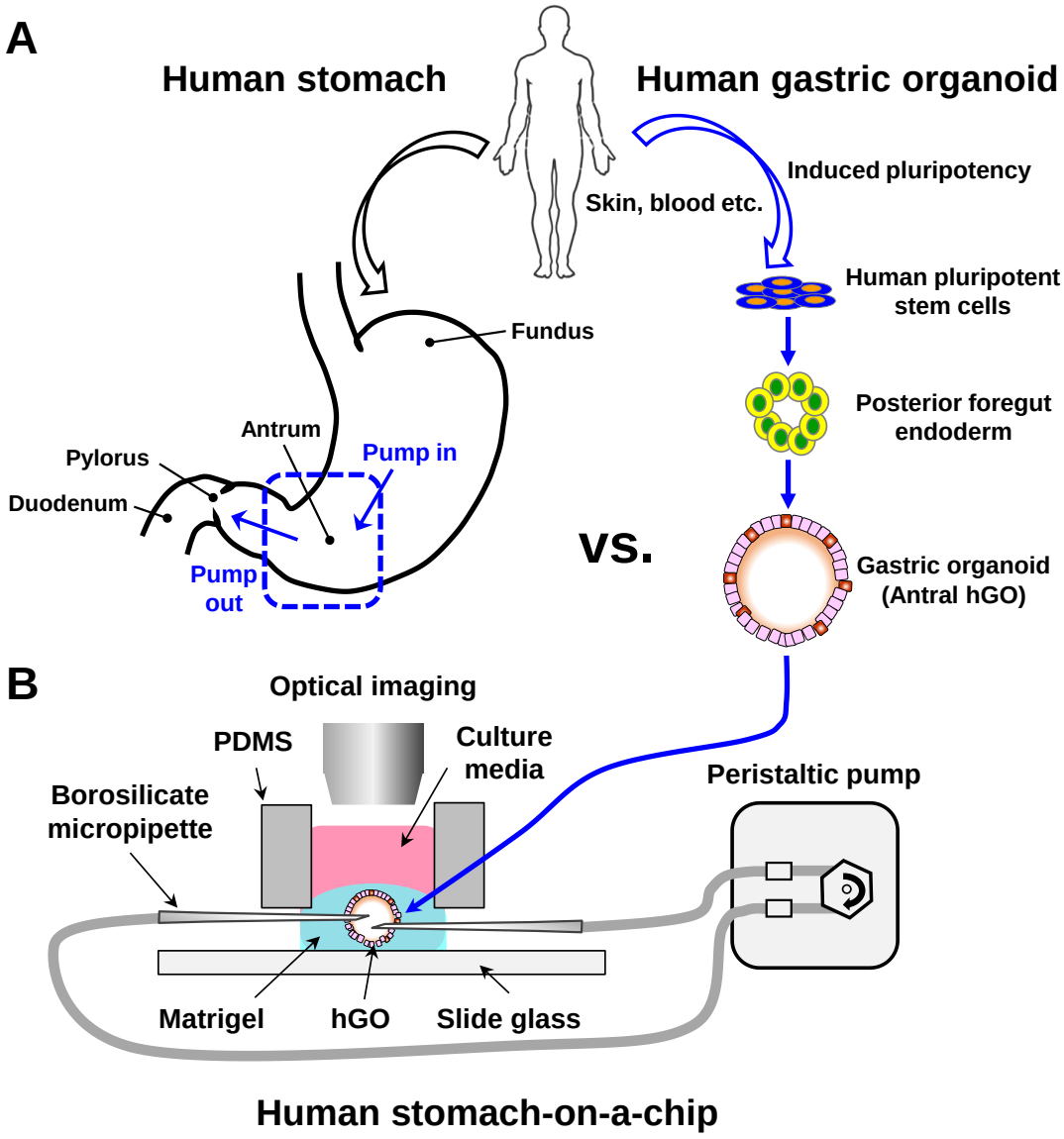
**Fig. 1.** Schematic overview of the proposed biomimetic human stomach-on-a-chip platform. (A) Human stomach versus antral hGO derived via differentiation of hPSCs. (B) Schematic diagram of the experimental setup for the generation of luminal flow in peristaltic stomach-on-a-chip.

**Fig. 2.** Fabrication procedure for human stomach-on-a-chip. (A) Schematic design concept for 3D printed mold. (B) Schematic design for human stomach-on-a-chip. (C) Fabricated PDMS-based human stomach-on-a-chip. (D) Human stomach-on-a-chip platform for luminal flow through hGO. The volume of media were ~ 450 μL in each in-line chambers, and ~ 50 μL above the hGO in Matrigel.

**Fig. 3.** Fluorescent time-course optical images of peristatic pumping through hGO within the stomach-on-a-chip platform. We observed expansions (4, 24, 43 sec) and contractions (17, 39, 50 sec) of hGO over time. This series recapitulates *in vivo* luminal flow.

**Fig. 4.** (A) Time-course images of fluorescent intensity (coded by 'Rainbow' LUTs) of steady-state peristatic pumping through hGO within the stomach-on-a-chip platform. Inclusion of air bubbles illustrates the steady-state flow through hGO over time, and demonstrates the pumping in (6, 33, 60 sec) and out (10, 44 sec) of the hGO. (B) Quantitative intensity measurements within a ROI adjacent to the hGO onto the stomach-on-a-chip platform. This graph was taken at an interval of 4 frames per second obtained from Fig. 4(A). Supplementary Mov. 2 represents one full rotation of the peristaltic pump motor. Periodicity in the fluorescence intensity corresponds to the mechanics of the three rollers of the peristaltic pump engaging and disengaging. The peaks of highest fluorescence intensity occurred at regular 34-second (1 cycle) intervals and equals one full rotation of the pump. When one roller engages and another disengages, there is a slight pressure fluctuation that causes a subtle expansion or contraction of the organoid. (C) We placed the ROI on the edge of the hGO when the pulsation caused the greatest expansion by brightfield. The graph was taken from a movie (300 sec) where the interval between frames was 4 frames per second. There are a series of peaks of intensities. The largest peak represents a single point of the peristaltic pump roller engaging the tubing. The lower peaks are caused by other rollers engaging the tubing. The time between the tallest peaks equals one rotation of the pump. This steady-state luminal flow over 30 minutes demonstrates proof-of-concept for long-term delivery and observation of luminal agents, illustrating biomimetic potential to model gastric chronic diseases.

**Fig. 1**


A
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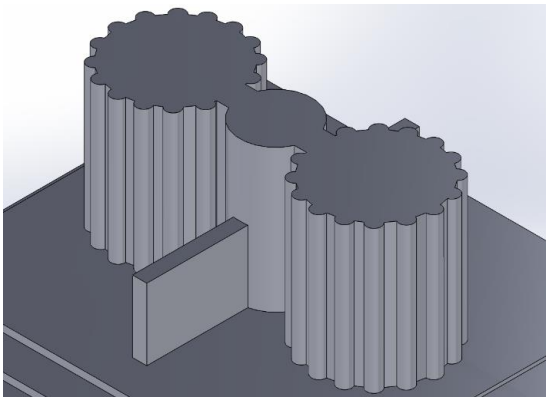
Human gastric organoid
Induced pluripotency
Skin, blood etc.
Human pluripotent stem cells
Fundus
Posterior foregut endoderm
Antrum
Pylorus
Pump in
Duodenum
vs.
Gastric organoid (Antral hGO)
Pump out
B
Optical imaging
PDMS
Culture media
Peristaltic pump
Borosilicate micropipette
Matrigel
hGO
Slide glass
Human stomach-on-a-chip

**Fig. 2**



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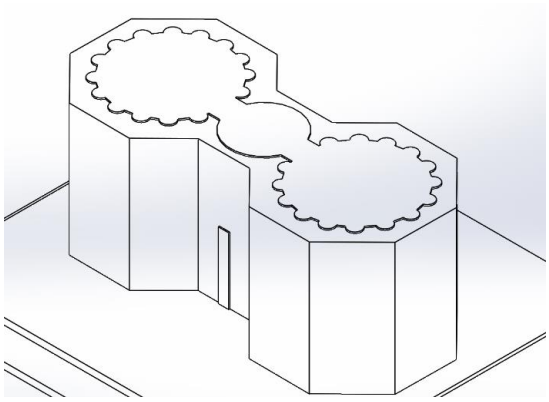

B


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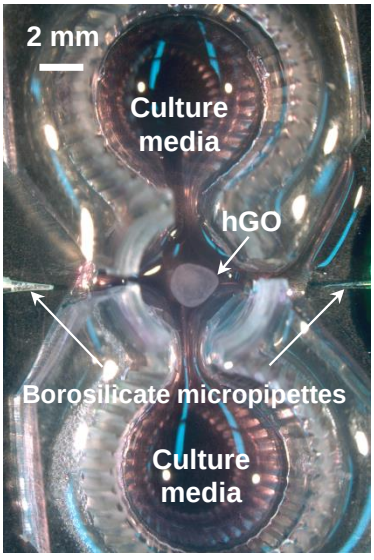
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Culture media
hGO
Borosilicate micropipettes
Culture media

D

**Fig. 3**

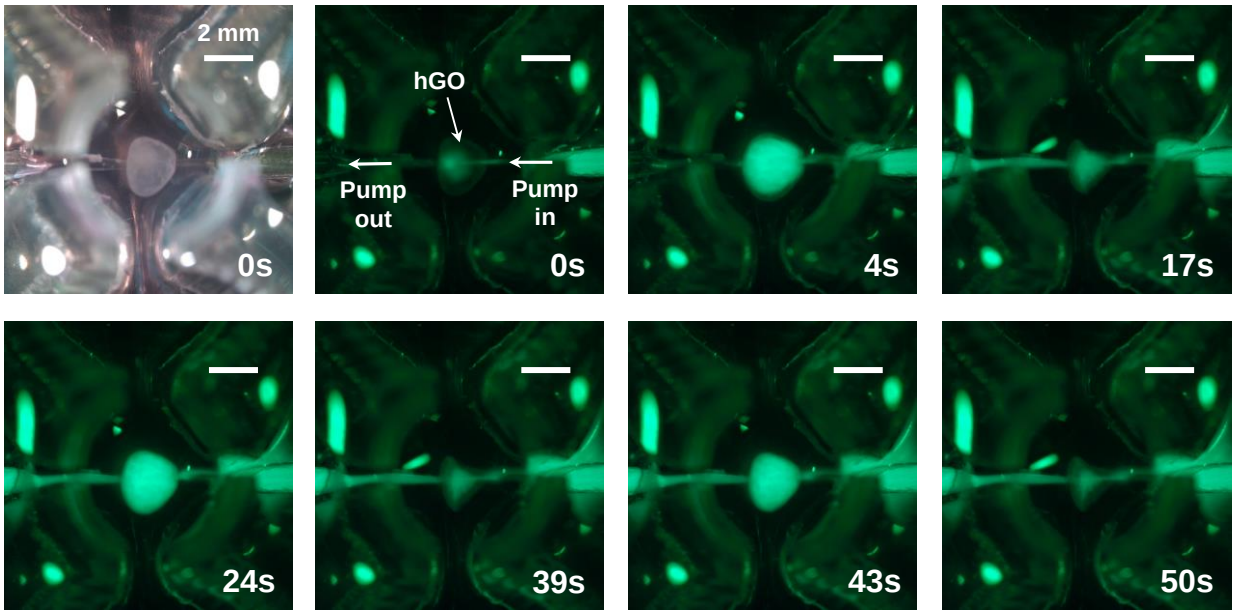
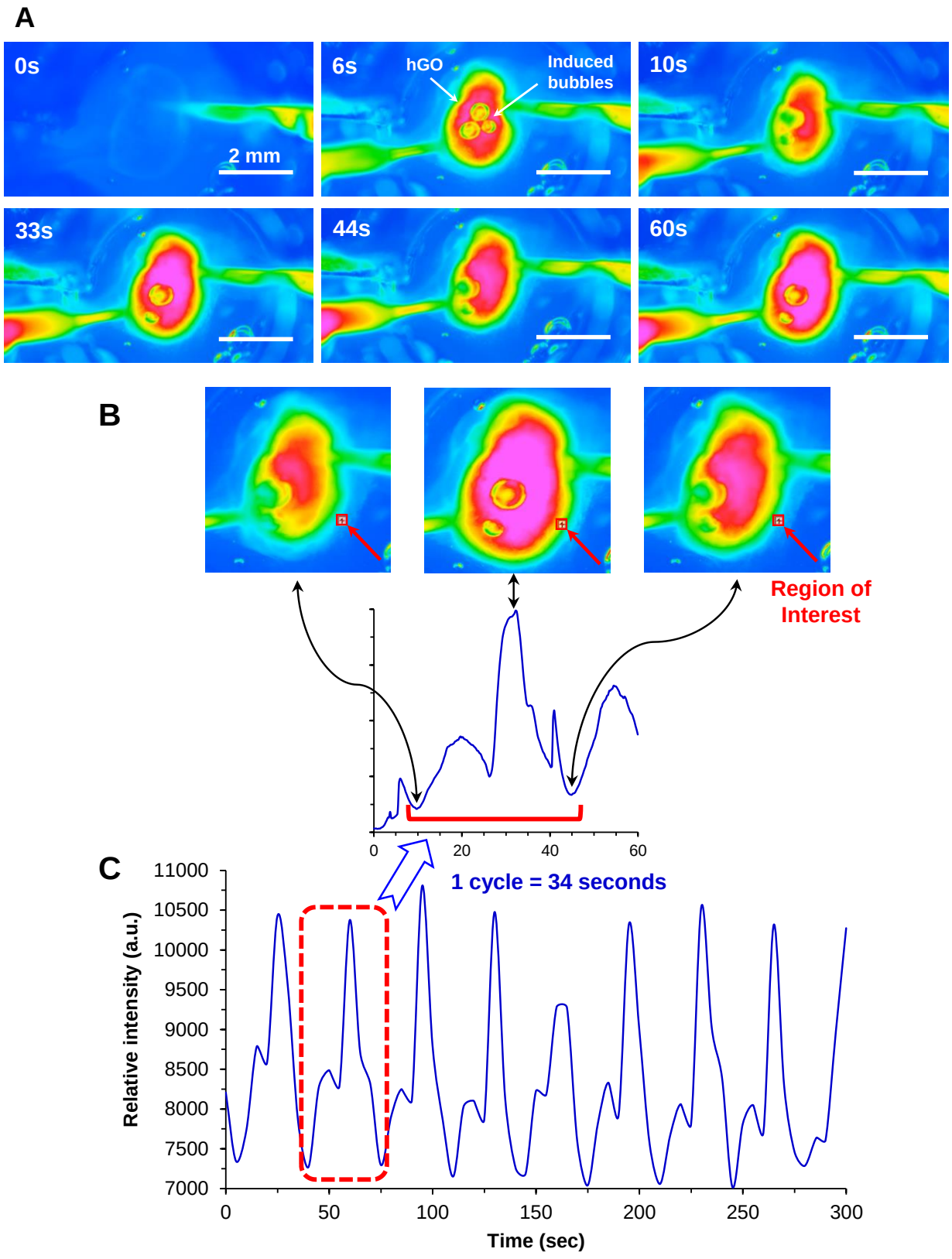


**Fig. 4**


A
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Relative intensity (a.u.)
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