AN INTEGRATED MICROFLUIDIC DEVICE FOR *C. ELEGANS* EARLY EMBRYOGENESIS STUDIES AND DRUG ASSAYS

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ABSTRACT

The *Caenorhabditis elegans* embryo is a widely used model for the functional analysis of dynamic cellular processes, such as chromosome segregation, cytokinesis or lineage analysis. However, the conventional embryo preparation method that relies on manually dissecting gravid worms to extract embryos is somewhat time consuming and does not lend itself to high throughput assays. Here, we report a fully integrated microfluidic approach for *C. elegans* early embryogenesis assays with unprecedented accuracy and throughput. The device consists of a compressible microfluidic pillar-array chamber for robust and fast on-chip extraction of embryos from the uterus of gravid nematodes. Subsequently, embryos are immobilized by automated fluidic transfer in a microtrap array for individual tracking of a large number of embryos, including fragile mutants with drug-permeable eggshells. Our device allows high-resolution live imaging of very early events in embryogenesis, starting from the one-cell stage. We also demonstrate the feasibility of well-controlled compound application in versatile microfluidic pharmacological assays performed on early embryos.

INTRODUCTION

The *C. elegans* nematode is a model organism of major importance in biomedical research related to human biology and disease [1]. In recent years, a number of microfluidic devices have been developed, for instance for behavioral or drug screening assays [2, 3]. These devices are generally designed for use with adult worms. The study of early *C. elegans* embryogenesis has played and continues to play an important role in the mechanistic dissection of fundamental cellular and developmental processes [4]. For the time being, early embryos are obtained by manual dissection of adult worms and subsequent transfer onto agarose pads prepared on a microscope slide. This protocol, whilst simple, comes with drawbacks, including being somewhat time-consuming, especially for beginners, and possibly perturbing embryo physiology, especially in drug assays that rely on the use of fragile embryos with permeabilized eggshells [5]. One group has proposed an improved approach where embryos were manually pushed on a microwell array after dissection [5]. Previously, our group introduced a microfluidic device for trapping of non-permeabilized embryos at an advanced development stage (> 40 cells) [6]. Here, we extend this concept and present a single integrated microfluidic device that allows harvesting and high-resolution optical imaging of permeabilized embryos, starting from the one-cell stage, as well as controlled drug application.

EXPERIMENTAL

Worm preparation

N2 wild-type *C. elegans* worms and worms expressing mCherry::histone2B, to mark chromatin, and GFP::PH, to mark cell membranes (strain GZ1326, generated by crossing strains OD57 and OD58 [7] from the Caenorhabditis Genetics Center), were used in this work. Strains were maintained at 24°C on nematode growth media (NGM) agar plates seeded with the auxotrophic *E. coli* strain OP50. In order to obtain embryos with a drug-permeable eggshell, the *perm-1* (T01H3.4) *C. elegans* clone was chosen from the Ahringer RNAi feeding library [8], and used to express dsRNA targeting *perm-1*. Bacteria were grown overnight at 37 °C in lysogeny broth medium (LB) with 100 µg/ml ampicillin. The culture was then diluted 1:10 in LB with 100 µg/ml ampicillin and grown at 37 °C until reaching an optical density at 600 nm (OD600) between 0.5 - 0.7 (*i.e.* after ~1 - 2 hour). The bacterial suspension was spread on standard NGM agar plates (6 cm, ~300 µl/plate) containing 0.01 mM isopropyl-β-D-thiogalactopyranosid (IPTG). Plates were dried in a sterile hood for ~1 hour and left at room temperature for ~24 hours to induce RNA expression. Subsequently, 20 to 30 N2 young adults were transferred onto each plate and incubated at 20 °C for 12 - 15 hours.

Chip fabrication

We used standard polydimethylsiloxane (PDMS) soft lithography techniques to fabricate the microfluidic chips (Fig. 1a). The master mold was made by a two-step process. First, patterns for all fluidic structures, in particular the trapping array and the foldable parts of the pillar array, were created on a silicon wafer by plasma etching (structure height 40 µm). Subsequently, a 20 µm thick SU-8 layer was spin-coated on the wafer and patterned to form the non-compressible pillar top pads, as well as to increase the total height of the main chamber and of the In1-Out1 fluidic path to 60 µm (Fig. 1a). The mold surface was treated with trimethylchlorosilane (TMCS) for 60 min to facilitate PDMS demolding. PDMS was poured over the SU-8/Si mold to form a 5 mm thick layer and cured at 80 °C for 1 hour. PDMS chips were plasma-bonded onto 0.17 mm thin glass coverslips for observation with high-resolution oil-immersion objectives (Carl Zeiss Axio Vert.A1, alpha Plan-Apochromat 100x/1,46 Oil DIC).

DEVICE DESIGN AND OPERATION

Our *C. elegans* embryogenesis device comprises two main functional components (Fig. 1a): (i) an embryo extraction chamber based on an array of compressible

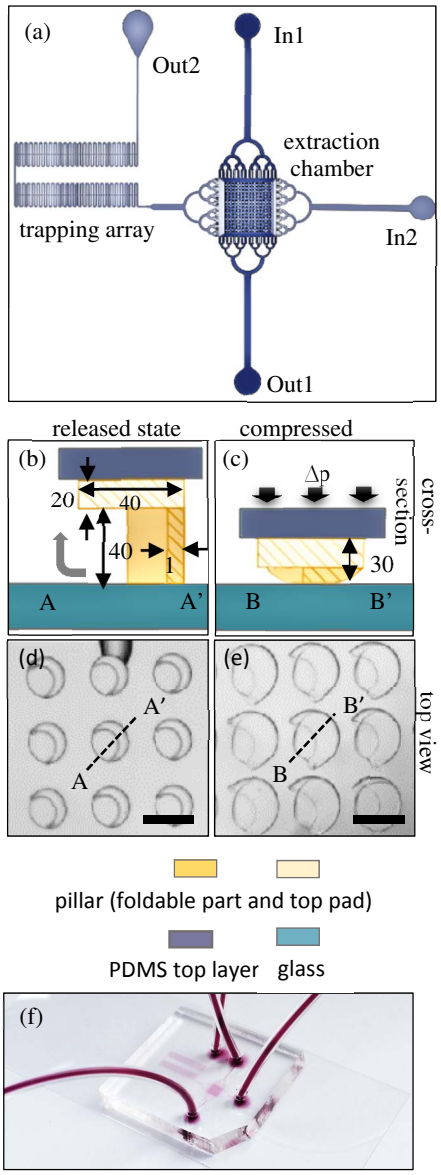

Figure 1: Microfluidic embryogenesis chip. (a) The integrated chip comprises an embryo extraction chamber with a reversibly compressible PDMS pillar array for extraction of embryos from gravid C. elegans worms, and an adjacent embryo trapping array. Dark violet patterns indicate 60 µm high and light violet patterns 40 µm high fluidic structures, respectively. The device has two fluidic inlets (In1, In2) and outlets (Out1, Out2). The chamber height is 60 µm in its released state, suitable to accommodate adult worms. (b,c) Cross-sectional views of a PDMS pillar (units are µm), built from a foldable crescent-shaped structure and a solid top pad, in its released (60 µm high) and in its compressed state (~30 µm high), respectively. The foldable part faces the glass substrate. Pressure is applied on the chamber roof (a 5 mm thick PDMS top layer, not to scale in the figure). (d,e) Top view images of a detail of the pillar array in the released (d) and compressed state (e), respectively. Scale bar = 50 µm. (f) Photograph of the PDMS-on-glass chip (20 mm × 20 mm) with fluidic connections. Microfluidics features are dyed in red.

PDMS pillars, and (ii) an embryo trapping array. Pillars of the array feature a specific dual-stage design with a 40 µm high foldable, crescent-shaped structure and a 20 µm high, barely compressible, cylinder-shaped pad on top of it. The pillar array is operated either in the released or in the compressed state (Fig. 1b and c). In the released state, the total pillar height is 60 µm, which is suitable to accommodate a population of adult *C. elegans* worms (body diameter ~60 µm). By compressing the chamber, the foldable part of each pillar collapses on its PDMS base, leaving a substrate spacing of about 30 µm (Fig. 1c). Photographs of the pillar array in either state and of the full PDMS chip are shown in Fig. 1d, e and f, respectively. To operate the device, first a suspension of 10 - 20 gravid worms is introduced in the extraction chamber through the In1-Out1 fluidic path (Fig. 2a and 2b). Mechanical expulsion of embryos from the worms' uterus is obtained by application of a brief manual pressure pulse to the chamber roof (Fig. 2c). The specific two-stage design of the PDMS pillar structures allows robust squeezing of adult worms without damaging the released embryos, whose minor axis diameter is ~30 µm. Subsequently, by activating flow along the In2-Out2 fluidic path, embryos are transferred into the microfluidic trapping array, where they are spontaneously immobilized by passive hydrodynamic action in a linear arrangement of dedicated microtraps (Fig. 3a). Each trap accommodates a single embryo (Fig. 3b). and drug compounds may be readily applied.

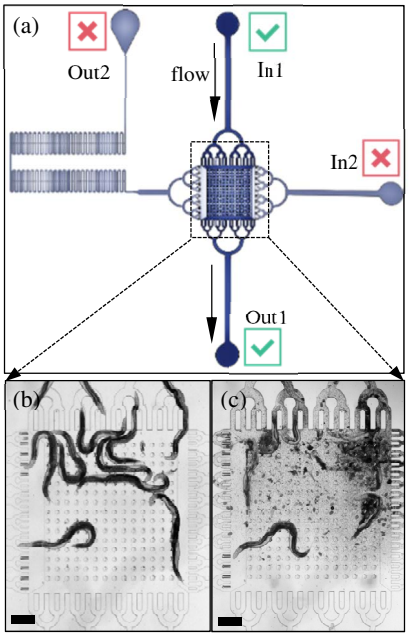

Figure 2: Protocol for on-chip embryo extraction. (a) Fluidic configuration for loading a suspension of adult worms into the extraction chamber (In1-Out1 open, In2-Out2 closed). (b) Image of worm loading. About 8 adult worms are in the chamber, which is in the released state. The PDMS pillar array and specific microfluidic filter structures at the chamber rims are visible. (c) Chamber after application of a pressure pulse from the top for squeezing the worms. A large number of extracted embryos populates the chamber. Scale bar = 200 µm.

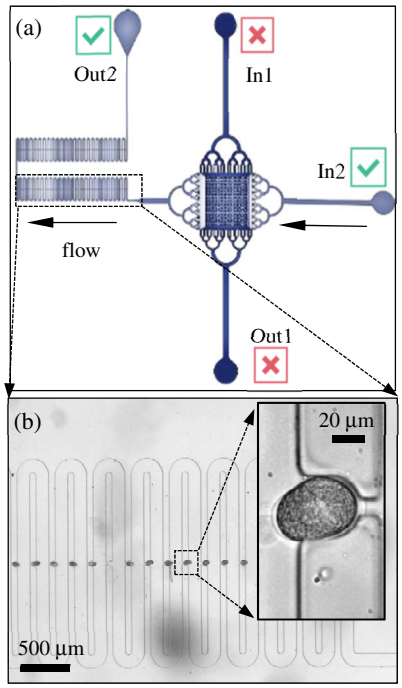

Figure 3: Microtrap array for embryo immobilization. (a) Fluidic configuration for embryo transfer from the extraction chamber to the trapping array, embryo immobilization and compound application (In2-Out2 open, In1-Out1 closed). (b) Serpentine microchannel with microtraps for hydrodynamic immobilization of embryos. The image shows a nearly completely filled portion of the array. The inset is a zoom on a one-cell embryo securely positioned in a dedicated microfluidic trap. Drug compounds may be applied through the serpentine channel in a well-controlled manner.

RESULTS

The embryo trapping array can receive up to 100 individual embryos, at development stages that correspond to the natural *in utero* distribution of the worms. The total time span from embryo extraction to imaging is as low as 1 - 2 min. Typically 5 to 10 one-cell embryos are captured per experiment and may be selected for further automated long-term imaging and analysis. Fig. 4 shows high-resolution brightfield images of normal cell divisions during initial *C. elegans* embryogenesis, *i.e.* from the one-cell to the 8-cell stage, confirming that on-chip environmental conditions are appropriate for this kind of assay [9]. Fig. 5 demonstrates on-chip drug treatment of an immobilized early embryo. Normal embryo development was observed prior to drug injection by fluorescent imaging. After 10 min, 10 µM Cytochalasin-D (Sigma-Aldrich), an actin polymerization inhibitor, was applied for 90 s through the microchannel of the embryo array, resulting in a failure of cytokinesis in embryonic blastomeres (Fig. 5, indicated by arrows). In this experiment, a single drug was applied at a specific embryo developmental stage and for a defined duration. We anticipate that, thanks to our microfluidic approach, drug screening assays with unprecedented versatility and control, including sequential application of different compounds or concentrations, can be implemented.

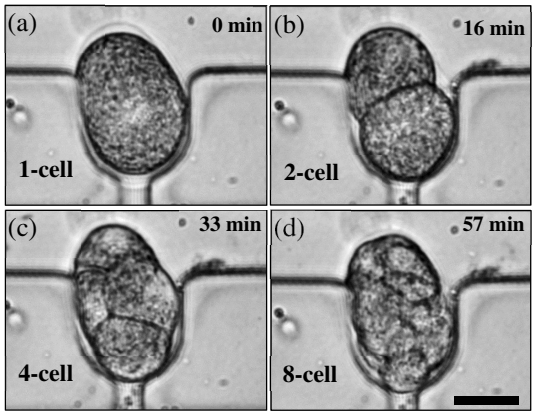

Figure 4: Live imaging of normal on-chip embryogenesis. Brightfield images (×63) of a microfluidic trap located in between two parallel sections of the serpentine channel in the trapping array (see Fig. 3). An immobilized C. elegans embryo is shown in the one-cell stage (a), and in subsequent 2, 4 and 8-cell stages (b-d). This sequence was taken over a timespan of about 1 hour (at time points indicated in the figure), demonstrating reliable embryo positioning over prolonged periods. Scale bar = 20 µm.

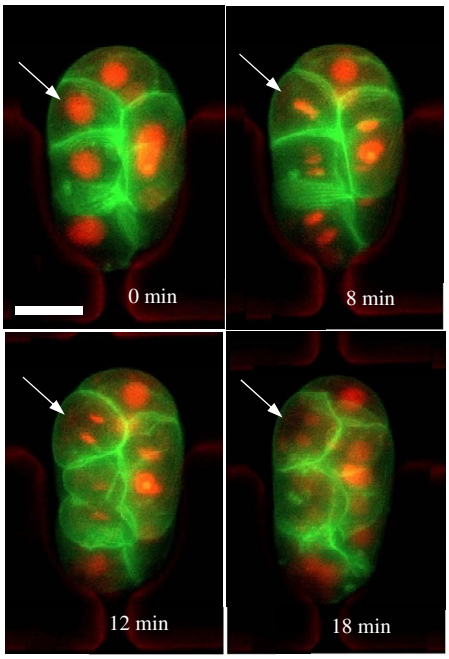

Figure 5: On-chip drug treatment in early embryogenesis. Sequence of fluorescent images of an 8-cell stage C. elegans embryo immobilized in a microtrap and rendered permeable by RNA-mediated depletion of perm-1 for 12 h at 20 °C (applied off-chip to the mother worm). Red mCherry::histone2B marks chromosomes and green GFP::PH binds to the plasma membrane. Cytokinesis failure was observed upon on-chip application of Cytochalasin-D for 90 s (at t ~ 10 min). The impact on a selected cell is highlighted by arrows, indicating that the cell progresses normally up to metaphase/anaphase (t = 0 and 8 min). Cytokinesis failed after drug application, leaving two nuclei in a single cell (t = 12 min). Subsequently, the two red chromosome signals become blurry (t = 18 min). Scale bar = 20 µm.

CONCLUSION

This article reports the first fully integrated microfluidic approach for *C. elegans* early embryogenesis studies. Our microfluidic chip is a powerful tool to realize on-chip embryo extraction and fast immobilization of fragile permeable embryos in a microtrap array. We demonstrate high-resolution live imaging of very early events in embryogenesis combined with well-controlled compound application through a microchannel directly to the point of interest. Such features present significant advantages with respect to conventional embryogenesis assays, in particular in terms of throughput, versatility and ease of manipulation.

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