MULTI-DIMENSIONAL IMAGING AND PHENOTYPING OF *C.ELEGANS* EMBRYOS *VIA* AN AUTOMATED MICROFLUIDIC DEVICE

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ABSTRACT

We introduce a microfluidic platform for automated on-chip worm culture, creation of synchronized embryo microarrays, and long-term parallel live imaging of *Caenorhabditis elegans* embryos. Our device allows studying well-defined embryo populations at unprecedented spatio-temporal resolution, via fully automated multi-dimensional imaging, covering six independent dimensions: the 3 spatial coordinates, development time, exposure duration/type (brightfield, fluorescent), and embryo number in the microarray. We successfully employed our platform to investigate the impact of perturbations of the mitochondrial functions on *C.elegans* embryogenesis. Our analyses revealed that specific mitochondrial stresses trigger a mitochondrial unfolded protein response (UPR^{mt}) in the embryos. These observations are the first proof that the UPR^{mt} molecular pathway is functional during *C.elegans* embryogenesis.

KEYWORDS: *C.elegans*, embryogenesis, microfluidic device, passive hydrodynamics, multidimensional imaging, automated phenotyping, UPR^{mt}, mitochondrial biogenesis

INTRODUCTION

The nematode *C.elegans* is one of the most employed model organisms in biomedical research [1]. In *C.elegans* many events occurring during larval stages are known to have a strong impact on the animal's lifespan. Whether conditions in the embryonic phase of life have an influence on the later development is, however, a much more challenging question to answer, mainly because systematic embryogenesis studies are extremely difficult from a technical viewpoint [2], since no technological tools for automated manipulation and analysis of *C.elegans* embryos are available yet. To solve this issue, we developed a new device for microfluidic handling of *C.elegans* embryos and creation of embryo microarrays for their automated imaging and phenotyping.

RESULTS AND DISCUSSION

Our microfluidic device, sizing 25 mm x 75 mm (standard microscope slide size), features lateral microfluidic connections, which make the device compatible for imaging with every upright or inverted microscope (Figure 1a). The chip design comprises two main components: a "worm culture chamber" (Figure 1b), where *C.elegans* nematodes are loaded, fed and treated, and an "embryo-incubator array" (Figure 1c), where the worms' progeny is transferred upon natural egg laying in the chamber. The whole system relies on pure passive hydrodynamics to perform a full set of automated operations, namely: (i) synchronization of the loaded worm population; (ii) worm culture and drug treatment; (iii) progeny transfer and embryo positioning in single micro-incubators; (iv) long-term embryo immobilization for high-resolution imaging. Specifically, a hydrodynamic trapping system (Figure 1d) has been designed for the reliable isolation of single *C.elegans* embryos with a trapping efficiency >99%. Upon arraying, embryos are retained in stable positions at 120 µm distance –i.e. under identical environmental conditions– for automated time-lapse observation and accurate phenotyping (Figure 1e).

By means of our device, we were able to discriminate embryonic development variations among different *C.elegans* transgenic and mutant strains at unprecedented resolution (Figure 2a-c). We then employed multi-dimensional imaging to study the spatio-temporal expression of the *hsp-6* protein in mutant worms affected by constitutive mitochondrial stresses (Figure 2d-i). Our analysis revealed the activation

of the mitochondrial unfolded protein response (UPR^{mt}) pathway [3], associated to a well-defined temporal evolution of *hsp-6* protein expression at specific locations within the embryonic tissues of the different mutants [4].

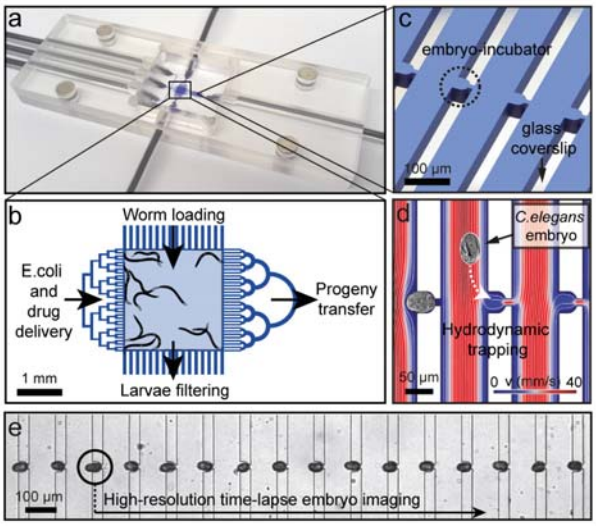

Figure 1: (a) Picture of the microfluidic device. (b,c) Schematic representation of the main constitutive parts of the chip: (b) the worm culture chamber – including a drawing of young adult C.elegans for size comparison – and (c) the embryo-incubator array. (d) Finite element method simulation (Comsol Multiphysics) of the fluid dynamics in the incubator array region, showing the principle of passive hydrodynamic arraying of single embryos. (e) Micrograph of a section of the array with immobilized embryos on which high-resolution time-lapse imaging is performed for automated embryo phenotyping.

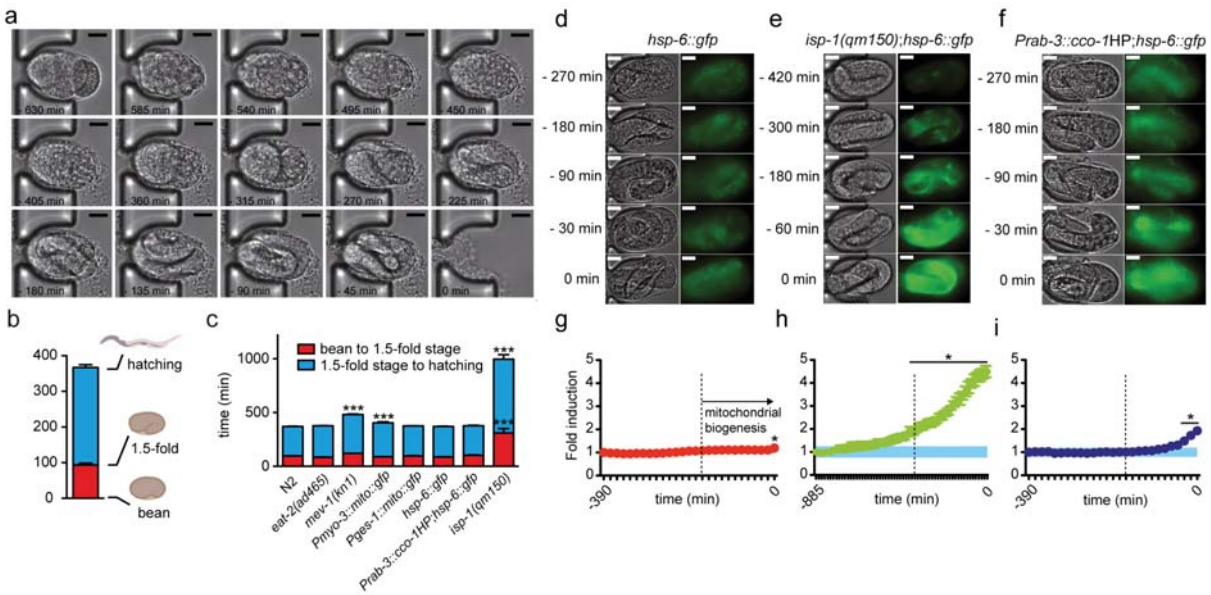

Figure 2: (a) Full embryonic development from egg capture in the incubator till hatching, as observed in a sequence of brightfield microscopy images (63× oil immersion objective, NA 1.4) taken from a movie (1 frame per minute) at 45 min intervals for the N2 wild-type worm strain at 25°C; the hatching time defines t = 0. Scale bars = 10 µm. (b) Average duration of different embryonic development phases – bean to 1.5-fold; 1.5-fold to hatching –, as observed for an array of 20 embryos of N2 wild-type worm strain at 25°C; (c) Analysis of duration of the embryonic development phases for different transgenic

*worm strains and mutants. Bar graphs are expressed as mean+SEM, *** $p \leq 0.001$. (d-f) Representative optical brightfield and corresponding fluorescent pictures that show the hsp-6::gfp expression in (d) wild type, (e) isp-1(qm150), and (f) Prab-3::cco-1HP strains. Scale bars = 10 μm. (g-i) Quantification of the hsp-6::gfp induction in (g) wild type, (h) isp-1(qm150) and (i) Prab-3::cco-1HP strains over the whole time-span from embryo capture to hatching (n=17, 7, and 21, respectively). The curves correspond to the relative GFP induction compared to the initial time point of each experiment. The blue area in each graph represents the base level 95% interval of confidence for each experiment.*

CONCLUSION

We developed a novel automated platform which allows accurately isolating synchronized populations of *C.elegans* embryos directly starting from an on-chip microfluidic worm culture. By means of our device, embryos can be studied in a fully automated way at extremely high spatial and temporal resolution. We demonstrated the capability of our platform to monitor the real-time dynamics of the embryonic development, to analyze protein expression in developing embryos, and to perform systematic studies on *C.elegans* embryos at unprecedented resolution and level of automation. We envision the widespread use of the proposed microfluidic technique for manifold protein expression and developmental studies at the embryonic level, as well as for investigating biological processes related to the aging mechanism and age-related diseases in particular.

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