

A microfluidic array for large-scale ordering and orientation of embryos

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Quantitative studies of embryogenesis require the ability to monitor pattern formation and morphogenesis in large numbers of embryos, at multiple time points and in diverse genetic backgrounds. We describe a simple approach that greatly facilitates these tasks for *Drosophila melanogaster* embryos, one of the most advanced models of developmental genetics. Based on passive hydrodynamics, we developed a microfluidic embryo-trap array that can be used to rapidly order and vertically orient hundreds of embryos. We describe the physical principles of the design and used this platform to quantitatively analyze multiple morphogen gradients in the dorsoventral patterning system. Our approach can also be used for live imaging and, with slight modifications, could be adapted for studies of pattern formation and morphogenesis in other model organisms.

Cell differentiation in embryos can be spatially controlled by the graded distribution of morphogens, chemical signals that act as dose-dependent regulators of gene expression. Some of the first morphogen gradients had been identified in the *Drosophila* embryo, in which dorsoventral patterning is initiated by the nuclear localization gradient of Dorsal (Dl). Dl is an NF- κ B transcription factor, which subdivides the embryo into three germ layers^{1–3}; the dorsoventral pattern of the embryo defines the dorsoventral pattern of the adult (Fig. 1a,b). The regions exposed to high, medium and low levels of Dl contribute to the formation of the mesoderm, the nervous system and the skin of the embryo, respectively.

Quantitative analysis of developmental systems controlled by morphogens requires information about both the regulatory regions of genes comprising the network and the spatial distribution of patterning signals. The dorsoventral patterning system in *Drosophila* is arguably one of the best understood systems with regard to its sequence-specific transcriptional regulation. However, information about the distribution of patterning signals is currently lacking, mainly because of technical difficulties associated with imaging the spatial distribution of proteins and transcripts along the dorsoventral axis of the embryo^{4,5}. When imaged on a regular microscope slide, embryos are oriented with their major axis parallel to the coverslip, and their dorsoventral

orientation is essentially random. As only a small fraction of embryos can be used for quantitative imaging, previous analyses of signals in the dorsoventral system relied on data collected from about ten embryos^{6,7}. To enable high-throughput analysis of the dorsoventral patterning signals, we developed a microfluidic embryo-trap array, a device in which hundreds of embryos are oriented vertically in a few minutes. Such ‘end-on’ orientation allows for dorsoventral-axis data to be easily collected for multiple embryos. Previously, end-on imaging has been possible only for very small numbers of embryos, which had to be individually and manually placed into an upright position^{5,6}.

Here we describe the design and the physical principles of the embryo-trap array, and use it to quantify morphogen gradients in fixed embryos and to monitor nuclear divisions in live embryos. The device enables high-throughput analysis of the dorsoventral patterning system at the level of the inductive cues and their signaling and transcriptional targets in multiple genetic backgrounds. Using this device to image a large number of embryos, we resolved an outstanding issue regarding the spatial extent of the Dl morphogen gradient.

RESULTS

Design of the embryo-trap array

The array is a one-layer microfluidic device fabricated from polydimethylsiloxane (PDMS), an optically transparent elastomer widely used in biological microfluidics^{8,9}. To allow for imaging of a large number of embryos, the array needs to have traps that are densely packed, which is an engineering challenge. Conventional approaches using hydrodynamics for cell trapping typically do not achieve such high packing density^{10,11} mostly owing to the requirement to properly balance flow resistance, resulting in a relatively large space between neighboring traps. The mechanism used in our design, in contrast, does not rely on resistance change upon the occupation of traps and therefore allows for dense arraying of ~700 traps in the space of a microscope slide (Fig. 1c,d).

Our design consists of a serpentine fluid-delivery manifold and an array of cross-flow channels (Fig. 1c,d). The 700- μ m-wide serpentine channel is wider than the major axis of the embryo (~500 μ m), allowing embryos of any orientation to move easily through it.

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