Laminar flows

Subcellular positioning of small molecules

Localized perturbation of processes that take place inside the living cell depends on molecular and spatial discrimination on a micrometre scale. Here we report the use of multiple laminar streams in a microfluidic channel to deliver membrane-permeable molecules to selected subcellular microdomains. This technique opens up avenues for non-invasively visualizing, probing and manipulating the cellular metabolic and structural machinery.

We have applied this method (called 'partial treatment of cells using laminar flows', or PARTCELL) to study the subcellular processes of mitochondrial movement and changes in cytoskeletal structure. Mitochondrial positioning is important for localized production of metabolic energy within different cellular microdomains, but analysis has so far been limited to imaging the movement of individual organelles. Local alterations in cytoskeletal architecture help to control cell shape, growth and motility[1,2], but investigation of these has previously had to rely on the exposure of whole cells to drugs that cause gross modification, such as complete disruption of actin microfilaments.

Microfluidic systems were prepared by placing a polydimethylsiloxane slab with channel features (300×50 μm) moulded into its surface onto a glass coverslip (Fig. 1a)[3–5]. By allowing different solutions to flow from the inlets at low velocity (about 0.6 cm s^{-1}), parallel streams of different liquids are created in the main channel (Fig. 1a, b)[4,6,7]. Under these conditions, there is no turbulence and the streams flow next to each other without mixing (apart from a small amount of diffusion). The width of each stream and the position of the interface between adjacent streams can be controlled by adjusting the relative amounts of fluid flowing in from each inlet. The interface is positioned between two adjacent streams — one with and the other without the molecule under investigation — over a single cell spread across the floor of the channel (Fig. 1b).

By streaming different solutions containing fluorescent tags over opposite poles of a live bovine capillary endothelial cell, we were able to maintain selective labelling of subpopulations of mitochondria in different regions of the cell (red and green in Fig. 1c, d) for over 2.5 hours, during which time the two subpopulations could be seen moving and intermixing inside the cell.

Figure 1e, f shows the disruption of actin filaments in selected regions of the cell after treatment with latrunculin A, a membrane-permeable molecule that binds to actin monomers and promotes the breaking up of polymeric actin filaments[8,9]. The disruption evident on the right of the cell, which was induced by targeted localization of latrunculin A, causes the mitochondria and the nucleus to shift to the left, although the peripheral shape of the cell remains relatively unchanged (Fig. 1f). This displacement of mitochondria, which are mainly associated with microtubules[10], by local disruption of actin microfilaments is consistent with the existence of mechanical connections between these two filament systems[2].

Our results indicate that partial treatment using laminar flows is an effective way to deliver small molecules to selected domains inside single mammalian cells. It is surprising that rapidly diffusing, membrane-permeable molecules can be directed so precisely, given the minute size of the cells themselves. Localization is achieved by a combination of rapid influx and efflux of molecules in distinct regions of the cell, created by using multiple laminar streams. Operation is straightforward and requires no special equipment apart from the moulds that form the capillary channels. Potential applications of our technique include the study of cell dynamics, chemotaxis, cell polarity, spatially regulated signalling, drug screening, and other phenomena that involve intracellular compartments and subcellular heterogeneity.

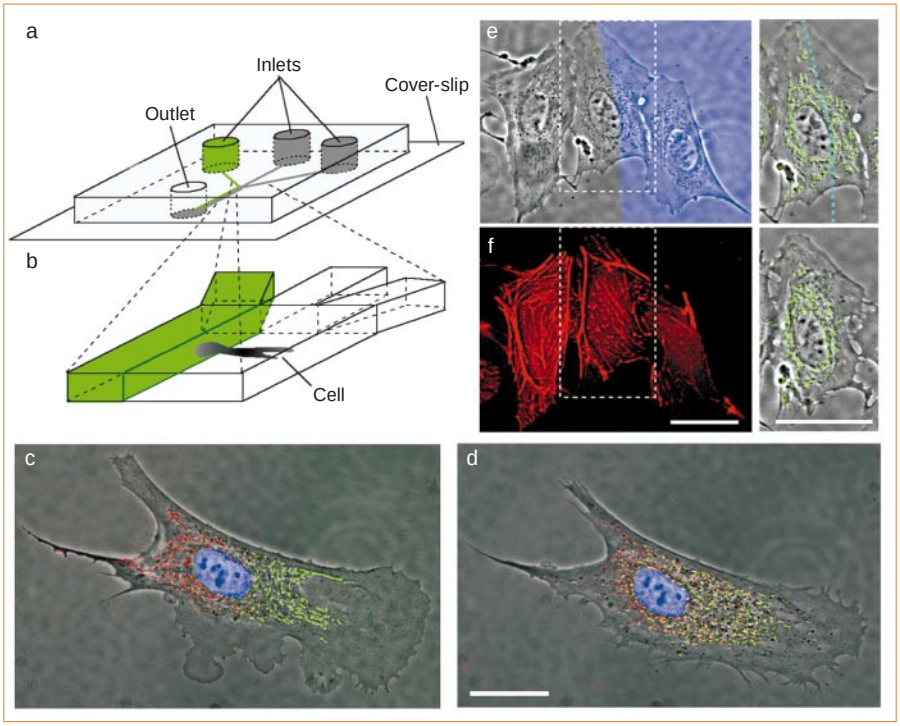

Figure 1 Differential manipulation of regions of a single bovine capillary endothelial cell using multiple laminar flows. **a, b,** Experimental set-up; **b** shows a close-up of the point at which the inlet channels combine into one main channel. **c,** Fluorescence images of a single cell after treatment of its right pole with Mitotracker Green FM and its left pole with Mitotracker Red CM-H_2XRos. The entire cell is treated with the DNA-binding dye Hoechst 33342. **d,** Image of the same cell as in **c** but taken 2.5 h later, showing intermixing of the red and green subpopulations of mitochondria. **e,** Treatment of a portion of a single cell with latrunculin A. The blue region (Dextran-70,000 Cascade Blue stain) reveals the flow of medium containing latrunculin A. Right, enlarged view of the middle cell and its mitochondria (green). **f,** Phalloidin–Alexa 594-labelled image of the same cell immediately after 10 min of treatment with latrunculin A. The cell in the middle, which was only partly in the stream containing latrunculin, shows disruption of actin microfilaments that is limited to this region (scale bars, 25 μm). Further details are available from the authors.

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