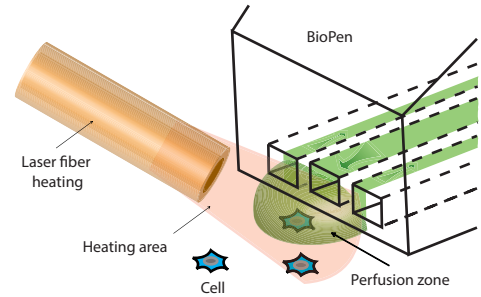


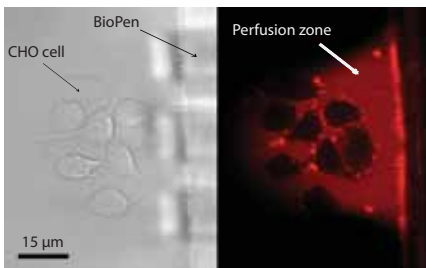
SINGLE-CELL ENZYMOLOGY

A novel approach for the study of single-cell intracellular enzyme activity at various temperatures was accomplished by using the BioPen in combination with a localized laser heating probe. A pore-forming agent α -hemolysin and an enzyme substrate fluorescein diphosphate (FDP) were delivered to individual cells by using the BioPen, to subsequently control the cell membrane permeability and target delivery of the FDP substrate. While adjusting the local temperature using a laser heating system, the activity of the enzyme alkaline phosphatase was monitored by following the production of the fluorescent product from the FDP substrate. The quantitative estimates for the intracellular alkaline phosphatase activity at 5 different temperatures in different cell lines were generated by constructing temperature-response curves of enzymatic activity at the single-cell level. Enzymatic activity was determined rapidly after cell permeation, generating five-point temperature-response curves within just 200 s.

Xu S. et al. A heating-superfusion platform technology for the investigation of protein function in single cells. *Anal. Chem.* (2015).



Using the BioPen in combination with a laser heating system to probe single-cell enzymology.



Using the BioPen to probe the ion channel TRPV1 permeability by exposure to various agonist solutions.

ION CHANNEL KINETICS

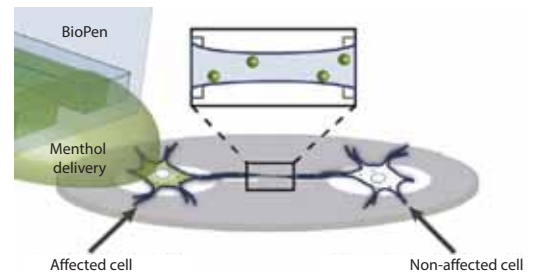
An adherent CHO cell culture, expressing TRPV1, was used to demonstrate the application of the BioPen for the delivery of ion channel agonist. More specifically, the effects of cholesterol depletion on the pore dilation of TRPV1 were investigated. The ion channels were activated with the agonists; capsaicin or protons (pH 5.5), and the ion-permeability properties of TRPV1 were assessed using whole-cell patch-clamp and YO-PRO uptake rate studies. While using the BioPen to locally perfuse agonists in tandem with YO-PRO, the uptake rate was monitored by measuring the intracellular fluorescence intensity using confocal microscopy. Cholesterol depletion was found to decrease TRPV1 mediated uptake rates of YO-PRO. Using localized perfusion enabled data libraries to be created from individual and small clusters of cells.

Jansson E. T. et al. Effect of cholesterol depletion on the pore dilation of TRPV1. *Mol. Pain* (2013).

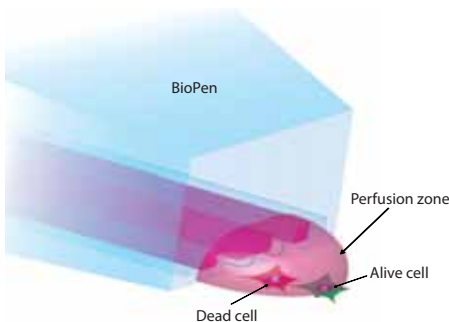
CELL-TO-CELL MOLECULAR TRANSPORT

The BioPen was utilized to target individual cells within interconnected networks, to investigate the cell-cell connections which spanned fabricated Teflon structures. Intercellular transport between HEK 293 cells, expressing the ion channel TRPM8, was measured by monitoring the changes in calcium concentration within individual cells. Menthol, in the presence of a Ca^{2+} spiked buffer solution, was delivered to one cell in a network to activate the TRPM8 ion channels. Utilizing the pre-fluorescent dye Calcium green-1, allows Ca^{2+} ion transport through the connections to be visualized and monitored. Measurement of the molecular transport, enabled the characteristics of cell protrusions crossing small microgaps to be established.

Zhang H. et al. Cellular communication via directed protrusion growth: critical length-scales and membrane morphology. *Nano Commun. Net.* (2015).



Using the BioPen to deliver menthol to only one of the connected cells to activate TRPM8 ion channels. The activation allows visualization of calcium transport through tubular interconnections.



Determination of individual cells viability by exposure to multi-component fluorescent agents using the BioPen.

SINGLE-CELL VIABILITY

A novel approach for determining the viability of individual cells in an adherent cell culture was developed by using the BioPen, in combination with a multi-component fluorescent response assay. Through directed exposure of the target cells to a pore-forming agent, the membrane permeability was controlled, where the extent of poration depended heavily on the cell type and life-cycle. The cell viability was able to be assessed 60 s after the poration, by simultaneous exposure to fluorescein diacetate and propidium iodide solution. Viable and non-viable cells were distinguished by the fluorescence emission of the two dyes within 10 s of the application. Hundreds of cells from four different cell lines, NG108-15, HEK 293, PC12, and CHO, were investigated within two days. These results demonstrate that the BioPen system is a facile, rapid, and reliable means to determine the viability in single-cell experiments.

Xu S. et al. A rapid microfluidic technique for integrated viability determination of adherent single cells. *Anal. Bioanal. Chem.* (2015).

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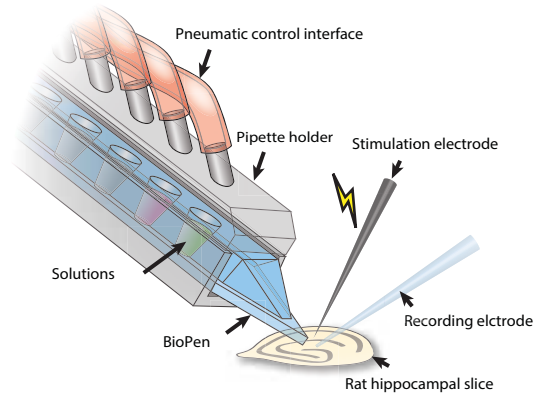
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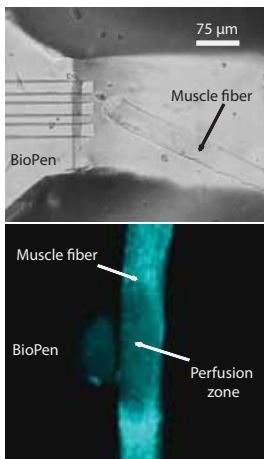
ISOLATED EXPOSURE IN BRAIN SLICES

The BioPen was used to administer pharmacologically active substances to selected areas in brain slices with high spatio-temporal resolution. The method utilized the hydrodynamically confined flow of the active chemical compound, to locally stimulate neurons, in conjunction with electrophysiological recording techniques. This enabled diverse superfusion experiments, testing the effects of different drug concentrations or candidates, on selected neurons in different cell layers with high positional accuracy. The technique was demonstrated in conjunction with electrophysiological recordings of pyramidal cells in hippocampal and prefrontal cortex brain slices from rats, to determine electric responses dependence with the delivery site. It was found that a multi-fold gain in solution exchange time could be achieved in comparison to whole slice perfusion. In addition, localized solution delivery by using the BioPen reduces the reagent consumption, while allowing more data to be collected from a single tissue slice, thus reducing the number of laboratory animals to be sacrificed for a study.

Ahemaiti, A. et al. A multifunctional pipette for localized drug administration to brain slices. *J. Neurosci. Methods* (2013).
 Ahemaiti, A. et al. Spatial characterization of a multifunctional pipette for drug delivery in hippocampal brain slices. *J. Neurosci. Methods* (2015).



Using the BioPen for local administration of biologically active substances to regions within brain slices.



Exposure of FCCP by using the BioPen to the end or side of a FDB fibre induces localized depolarization.

SINGLE MUSCLE FIBER PHYSIOLOGY

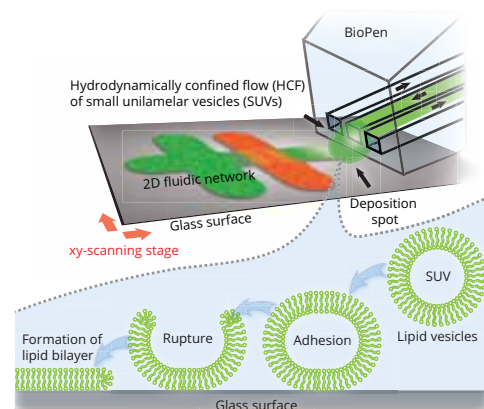
Using the BioPen, compounds were delivered locally to the end or side of single adult mouse skeletal muscle fibres to test whether changes in mitochondrial membrane potential were transmitted to more distant located mitochondria. Mitochondrial membrane potential was monitored with tetramethylrhodamine ethyl ester (TMRE). Cytosolic free $[Ca^{2+}]$ was monitored with fluo-3. A pulse of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) applied to a small area of the muscle fibre (30 μm in diameter) which produced a rapid decrease in the mitochondrial TMRE signal (indicative of depolarization) to 38% of its initial value. After washout of FCCP, the TMRE signal partially recovered. At distances greater than 50 μm away from the site of FCCP application, the mitochondrial TMRE signal was unchanged. Similar results were observed when two sites along the fibre were pulsed sequentially with FCCP. After a pulse of FCCP, cytosolic $[Ca^{2+}]$ was unchanged and fibres contracted in response to electrical stimulation. These results indicate that extensive networks of interconnected mitochondria do not exist in skeletal muscle. Furthermore, the limited and reversible effects of targeted FCCP application with the BioPen highlight its advantages over bulk application of compounds to isolated cells.

Bruton, J. et al. Usage of a localised microflow device to show that mitochondrial networks are not extensive in skeletal muscle fibres. *PLoS One* (2014)

BIOMEMBRANE PRINTING

Lipid bilayer membranes are among the most ubiquitous structures in the living world, with intricate structural features and a multitude of biological functions. It is attractive to recreate these structures in the laboratory, as this allows mimicking and studying the properties of biomembranes and their constituents, specifically exploiting the intrinsic two-dimensional fluidity. The BioPen was used as a rapid prototyping technology for two-dimensional fluidic platforms, based on *in-situ* generated circuits of phospholipid films, by deposition and fusion of small unilamellar vesicles of varying compositions. In this "lab-on-a-biomembrane", various chemical and physical operations, such as writing, erasing, functionalization, and molecular transport, could be applied to user-defined regions of a membrane circuit. The BioPen system operates in an "open volume", i.e., outside the confinement imposed by channels and chambers, providing unique opportunities for interaction with biological samples.

Ainla, A. et al. Lab on a biomembrane: rapid prototyping and manipulation of 2D fluidic lipid bilayers circuits. *Sci. Rep.* (2013).



Using the BioPen to write 2D nanofluidic networks composed of supported phospholipid membranes and dynamically modify their connectivity, composition and local function.