**Microfluidic multimolecular delivery for personalized medicine**

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**Introduction** Variation in treatment responses remains a formidable obstacle when selecting ideal therapies for individuals suffering from various diseases. Although systematic and quantitative assays performed directly on cells harvested from the patient are ideal to tailor treatment plans and to unveil factors conferring interpatient variability in drug responses, the requirement for primary cell assays poses a particular challenge for translation into the clinic because collection, testing, and manipulation of these fragile and rare cells are costly, time-consuming, and labour-intensive. To address these shortcomings, we developed the therapeutic agent screening microfluidic electroporator (THEME), capable of performing combinatorial drug screenings directly on cells purified from blood in integrated sequential steps.

**Methods** THEME was constructed by integrating previously developed vortex-mediated cancer cell purification mechanism1 and multimolecular delivery system22. We also designed a compact and programmable custom-built pulse generator, composed of a crystal regulated microprocessor (MSP430F5529, Texas Instrument), opto-couplers, a three stage H bridge, to generate 20kHz square pulses with 50% duty cycle for 1ms duration and 1s burst interval with a set voltage amplitude. The gefitinib-resistant clone of non-small cell lung cancer cells (HCC827 GR6) 3 were spiked into 20X diluted whole blood to emulate circulating tumor cells from gefitinib-resistant lung cancer. A membrane impermeable propidium iodide (PI) was delivered into cells to identify the optimum electroporation condition. Combinatorial drug efficacy on restoration of drug sensitivity was evaluated by injecting gefitinib (EGFR inhibitor) and PHA-665752 (MET inhibitor3) into HCC827 GR6 cells purified from blood via electroporation in a dose-controlled manner. The processed cells were cultured for 72hrs in 96-well plates and CellTiter-Glo® luminescent assay (Promega) was performed to quantify dose-dependent toxicity of drug combinations. Relative viability of processed cells was normalized by that of untreated controls.

**Results** Automated solution exchange scheme of THEME along with real-time visualization capability enabled dose-controlled combinatorial drug screening on cancer cells purified from blood without laborious and time-consuming pipetting, centrifugation and imaging steps. The collected samples contained low number of assay-interfering blood cells. The high efficiency (96%) and viability (97%) were observed for HCC827 G6 cells when 7 trains of 12Vrms square pulses were applied.

**Discussion** While HCC827 GR6 cells, regardless of electroporation, exhibited similar degrees of dose-independent drug resistance to both Gefitinib and PHA-665752 when single-drug was administered, cells treated with Gefitinib via electroporation reached the endpoint cytotoxicity within 24hrs, suggesting THEME’s potential as an accelerated drug efficacy testing platform. Combinatorial administrations of two drugs restored drug sensitivity of HCC827 GR6 cells and electroporated cells exhibited higher re-sensitization tendency at a lower dose. This could be attributed to higher in-cell drug concentrations achieved by electroporation-mediated drug delivery, suggesting that THEME can be used to identify the drug efficacy variations sensitive to subtle changes in the concentration.

**Conclusion** THEME has a great potential for patient-specific drug selections and efficacy evaluation by directly assaying primary cells purified from bodily fluids.

**References**

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