GENOME-LEVEL MAMMALIAN CELL RESPONSES TO DIGITAL MICROFLUIDIC ACTUATION

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ABSTRACT
Digital microfluidics uses electric fields drive droplets, often containing living cells, across a hydrophobic surface. We present work characterizing the effects of DMF actuation on cells at the genome-level. Cells actuated under standard operating conditions at a low frequency experienced little-to-no DNA damage and small changes to gene expression, while cells actuated at ~2 times the standard voltage, ~3 times the standard operation time, on ~10 times the standard electrode areas and at ~10x the standard frequency experience DNA damage and changes to gene expression. We propose that these results form a useful metric for the growing number of scientists using digital microfluidics and cells.

KEYWORDS: Digital microfluidics, cell stress, heat shock, DNA damage

INTRODUCTION
Digital microfluidics (DMF) is a fluid-handling technique in which nanoliter to microlitre droplets can be manipulated by electrically charging electrodes embedded under a hydrophobic insulating layer. DMF has recently emerged as being a useful tool for miniaturized culture [1] and analysis [2] of mammalian cells with reduced quantities of consumables and improved sensitivities relative to conventional techniques [3]. For a droplet manipulated by DMF, the vast majority of the electric field drops across the protective insulating layer; however, it is still important to characterize the potential effects of DMF manipulation on cells because electrical fields are known to affect cell behaviour and viability [4]. Cell stress responses have been previously characterized for cells manipulated under dielectrophoretic frequencies [5] (~0.1-100 MHz), but we are unaware of any such studies for cell manipulation under conditions associated with digital microfluidics (typically ~1 kHz fields across a thick insulating layer). We report here the first genome-level investigation of the effects of DMF actuation on cells.

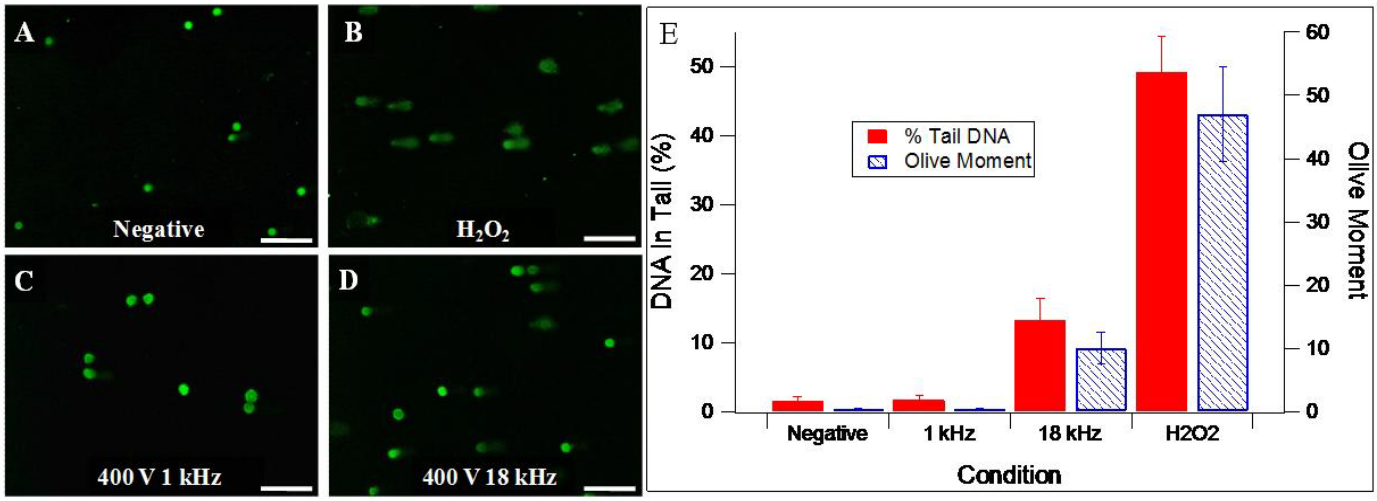

Figure 1: Representative photomicrographs of BA/F3 cells assayed for DNA damage using the single cell gel electrophoresis (COMET) assay. Cells were either untreated (A), hydrogen peroxide treated (B), actuated continuously for 15 minutes at 400 Vpp 1 kHz (C) or 400 Vpp 18 kHz (D) on digital microfluidic devices. Scale bar is 50 µm. Percent fragmented DNA (solid red bars) and Olive moment (striped blue bars) were quantified by CometScore™ (E)

EXPERIMENTAL Devices bearing 10 mm wide square electrodes arranged in 2x2 arrays were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) fabrication facility using photolithography and wet etching. Devices were assembled with an unpatterned ITO–glass top plate and a patterned bottom plate separated by a spacer formed from four pieces of double-sided tape (total spacer thickness 280 μm). For cell actuation experiments, 70 µL droplets of RPMI-1640 supplemented with 10% fetal bovine serum and 0.06% pluronic F88 (wt/v) volume containing 2 x 10^6 cell/mL Ba/F3 pro-B murine immortalized suspension cells were actuated continuously for 15 minutes (approximately 3-fold longer than DMF manipulation in most experiments) in a circular pattern on devices at a driving voltage of 400 V_{pp} (approximately 2-fold greater than typical potentials) at frequencies of 1 kHz or 18 kHz. Cells were diluted in 1 mL fresh media and allowed to recover for 1 hour at 37°C before RNA extraction and analysis. Untreated

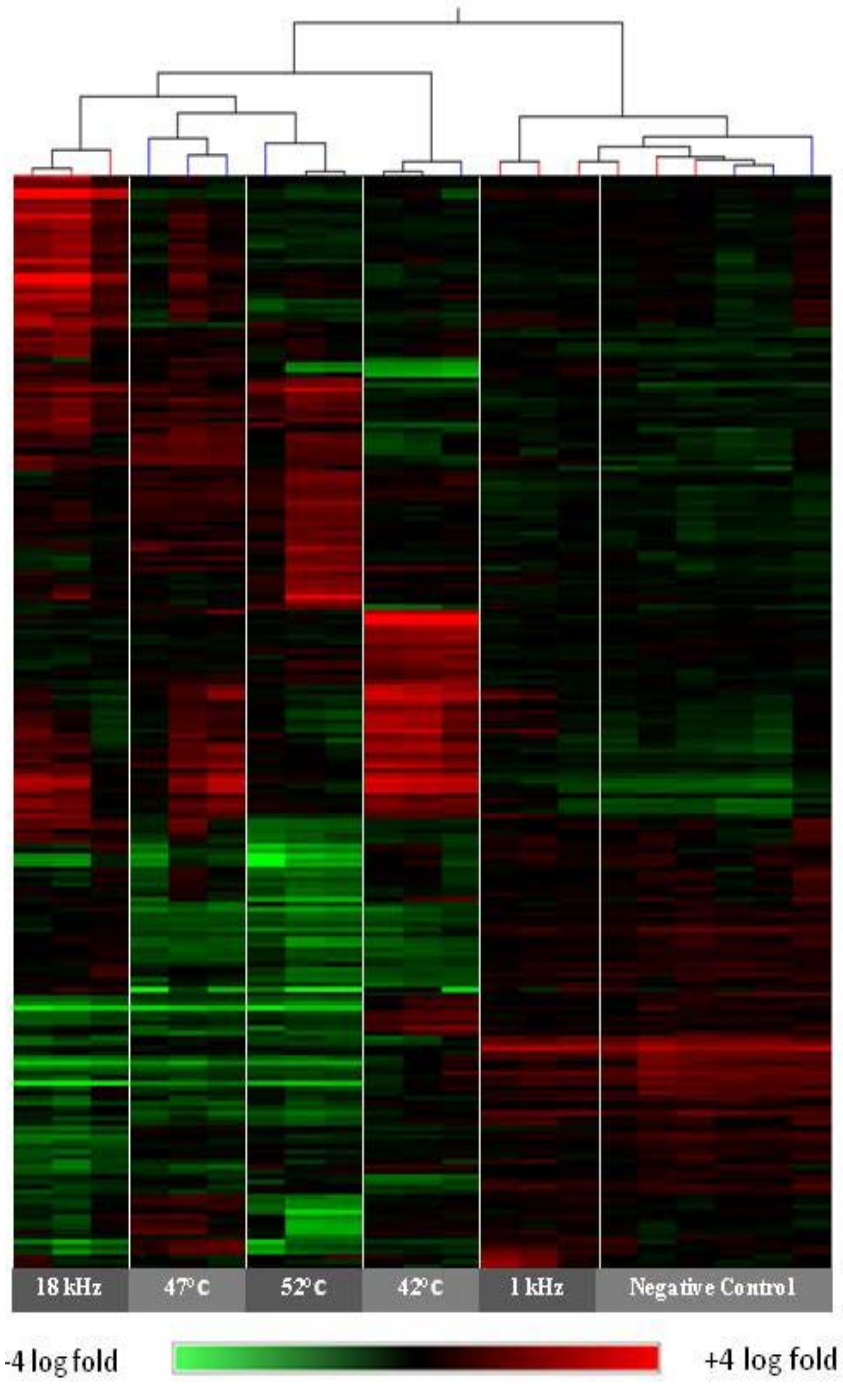

Figure 2: Heat map of significant probes with ≥2 absolute fold change cut-off by ANOVA using FDR Benjamini and Hochberg multiple testing correction ($p<.05$). Cells were manipulated for 15 minutes by DMF at 400 Vpp and frequencies of 18 kHz or 1 kHz, or externally heated on chip to 42°C, 47°C or 52°C. Trees were generated with a Pearson centered correlation tree building algorithm as a distance metric with average linkage rules. Green shaded cells represent log fold-change downregulated expression and red shaded cells represent log fold-change upregulated expression versus the mean.

control cells were treated similarly on device, but were not subjected to driving voltages. Heat shock control cells were heated on device with a hotplate and the center temperature of droplets controlled to within 1°C of set-point by measurement with a K-type KMQSS-010U-6 thermocouple (Omega Engineering, Inc.). COMET assays were conducted using a Comet Assay Kit (Trevigen, Inc.) and quantified using Cometscore™ (AutoComet), reverse transcription was completed using a Quantitect Reverse Transcription Kit (Qiagen, Inc.) and PCR was conducted on a 7900HT qRT-PCR machine (Applied Biosystems, Inc.) using Quantifast SYBR green PCR kit (Qiagen, Inc.). cDNA microarrays were conducted on the BeadChip platform (Illumina, Inc.) by the University Health Network Microarray Centre.

RESULTS AND DISCUSSION

Previous studies evaluating electrical-field mediated cell damage have reported observing DNA strand breaks [7]. To detect DNA fragmentation in cells manipulated by DMF, we conducted Single Cell Gel Electrophoresis COMET assays, with DNA damage quantified by percentage of migrating DNA and the Olive moment. Non-actuated cells and cells actuated at 1 kHz had very little DNA migration/damage(Figure 1A/C/E), and significantly less than cells treated with H_2O_2 or cells actuated at 18 kHz (Figure 1B/D/E).

To broadly probe for phenotypic changes, we conducted full-genome microarray studies on heat treated cells, and non-actuated and actuated cells (as above) using Illumina BeadChip Arrays. Figure 2 is a heat map of all significant genes with ≥2 absolute fold change as determined by one way analysis of variance (ANOVA). Of the 221 differential expressed genes ($p<0.05$) only 3 were statistically different between the non-actuated cells and the cells actuated at 1 kHz. However, there were 92 were statistically different probes between cells treated at 18 kHz vs. untreated controls, many of which included genes involved in the p38 MAPK, JNK-STAT and fos/jun pathways. Furthermore, the similarities between the expression profiles of cells actuated at 18 kHz vs. the heat shock controls suggest that heating may contribute to any changes in gene expression brought on by DMF manipulation.

Table 1: Summary of qPCR results. Fold changes in expression and associated p-values derived from Student's t-test for dusp1 and hspd1 genes of cells actuated on DMF devices for 15 minutes with square electrode sizes of 10, 5 mm and frequencies of 18, 10, 1 kHz.

	Dusp1		hspd1	
Condition	**Fold Change**	**p-value**	**Fold Change**	**p-value**
10 mm 18 kHz	-3.52	<0.01	-3.17	<0.05
10 mm 10 kHz	-1.03	>0.50	-1.46	<0.30
10 mm 1 kHz	-1.27	<0.20	-1.04	>0.50
5 mm 18 kHz	1.43	>0.50	1.05	<0.20
5 mm 10 kHz	1.44	<0.10	1.73	<0.20
5 mm 1 kHz	1.04	>0.50	1.05	>0.50

Two genes were then chosen for qRT-PCR analysis to verify the microarray results and to characterize the influence of frequencies (1, 10 and 18 kHz) and electrode sizes (5x5 mm, or 10x10 mm – both of which are larger than usual). Dual specificity phosphatase 1 (dusp1) and heat shock 60kDa protein 1 (hspd1), which were both downregulated from the microarray at 18 kHz, were examined (Table 1). Of all the conditions, only the case of high frequency (18 kHz) and large electrodes (10 mm x 10 mm) led to significant decreases in dusp1 or hspd1 expression levels ($p<0.05$).

CONCLUSION

In conclusion, it appears that DMF operating parameters can be tailored to generate mammalian cell stress responses that range from negligible to severe; we propose that these results will be important for the increasing number of groups using DMF for cell-based applications.

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