

## Supplementary Information for:

A new angle on Pluronic additives: advancing droplets and understanding in digital microfluidics

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## DMF Device Longevity Analysis

A longevity assay was developed to evaluate the potential for Pluronic additives to increase device lifetime. The bottom plate of the device used for this assay featured a linear array of 3 square (4×4 mm) actuation electrodes with inter-electrode gaps of 30 μm. Devices were assembled with a patterned bottom plate and an unpatterned ITO–glass top plate separated by a spacer formed from 2 pieces of double-sided tape (total spacer thickness 140 μm). To actuate droplets, driving potentials (200 V<sub>pp</sub>) were generated by amplifying the output of a function generator (Agilent Technologies, Santa Clara, CA) operating at 5 kHz. Droplets were sandwiched between the two plates and actuated by applying driving potentials between the top electrode (ground) and sequential electrodes on the bottom plate via exposed contact pads on the bottom plate. Droplet actuation was monitored and recorded by a CCD camera mounted on a lens.

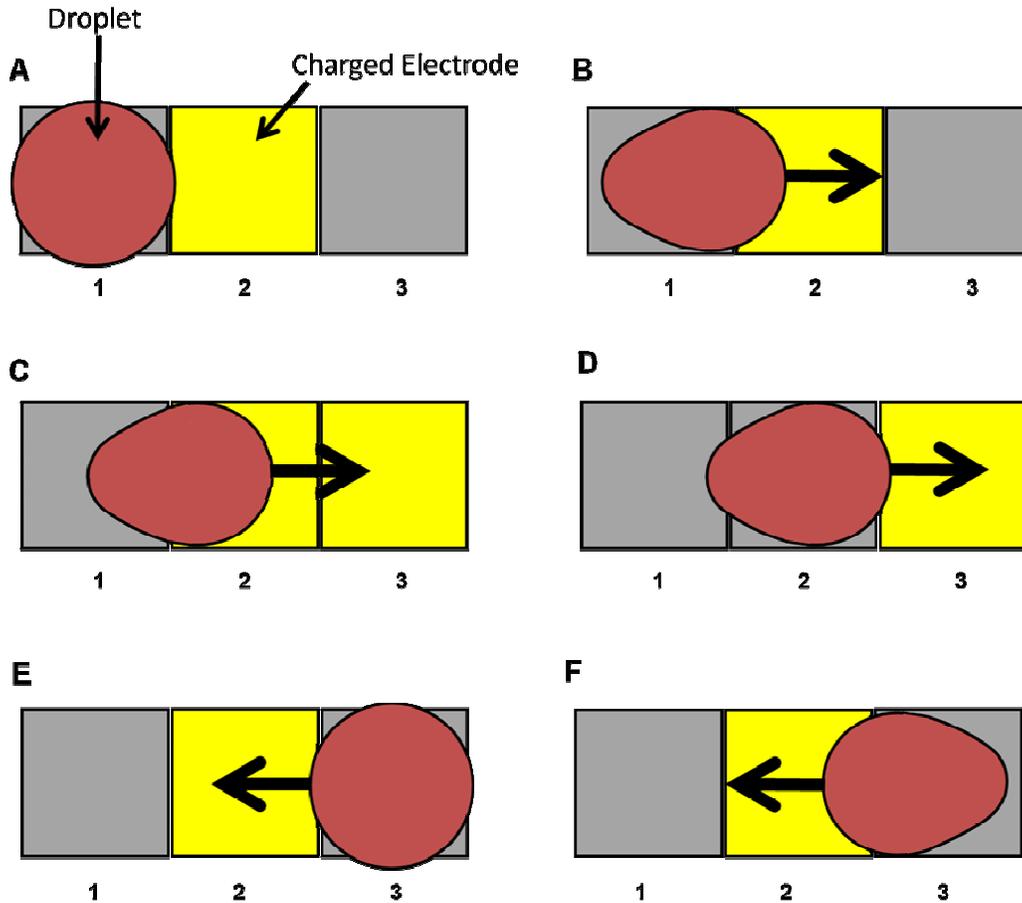
The longevity assays were used to evaluate RPMI 1640 cell culture medium with 10% fetal bovine serum (FBS) (Life Technologies/Invitrogen Canada, Burlington, ON) containing one of eight Pluronic additives at a concentration ranging from 0.0 to 0.15% (w/v). Each concentration was evaluated 3 times on 3 different devices. During each assay, a 4-μL droplet was actuated in a five-step process as depicted in Figure S1. Briefly, (step 1) with a droplet over electrode 1, electrode 2 was charged to initiate droplet motion (Figure S1A/B). (Step 2) Once the droplet had travelled to the middle of electrode 2, electrode 3 was charged (while electrode 2 remained charged) (Figure S1C). This ensured smooth droplet transitions between electrodes without pause. (Step 3) Once the droplet had travelled onto electrode 3, the potential was removed from electrode 2 (Figure S1D). (Step 4) The potential was removed from electrode 3 once the droplet reached the end of the electrode, and the droplet was moved back to its original position (Figure

S1E-F). (Step 5) Steps 1-4 were repeated in the same manner reversing directions at the end of each cycle until the device failed. As droplets containing cell culture media were actuated across electrodes in this manner, the speed of the droplets was observed to decrease over time. Device failure was defined as any case in which a droplet required more than 15 seconds to complete a movement step from one electrode to the next. The number of steps and the time until device failure were recorded for each condition.

For concentration dependent studies, the actuation times  $t$  as a function of Pluronic concentration in cell media  $c$  were fit to a lognormal curve (Eq. 1):

$$t = t_0 + A e^{\left[ \frac{(-\ln(c/c_0))^2}{width} \right]} \quad \text{Eq. 1}$$

where  $t_0$ ,  $A$ ,  $c_0$  and  $width$  are constants.



**Figure S1:** Schematic of continuous actuation of cell culture media for device lifetime assays. Grey squares represent uncharged (non-potentiated) electrodes and yellow squares represent charged (potentiated) electrodes. In (A), an electrical potential is applied to electrode 2 to initiate droplet motion. In (B), the droplet begins to move onto electrode 2. In (C), when droplet is halfway over electrode 2, a potential is applied to electrode 3 to ensure continuous droplet motion. In (D), the potential is removed from electrode 2 once droplet reaches electrode 3. In (E), once droplet has moved to the end of electrode 3, a potential is applied to electrode 2 to change the direction of movement. In (F), the droplet begins to move across electrode 2.