Constraints of cell growth in narrow microchannels

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Abstract

Cell growth is fundamental to many processes in basic and applied biology. Growth rates afford a window into how cells respond to differing environmental and nutritional cues. Typically measured at the population level at scales of culture tubes, culture flasks and shake flasks, growth rates at the single cell level has emerged to be of keen scientific interest with the advent of techniques for handling and interrogating single cells. Writing in *Nature Biotechnology*, Manalis and coworkers outline a microfluidics approach for measuring growth rates of different types of cells at the single cell level. Specifically, the approach entails flowing single cells through micrometer-sized microfluidic channels, where passage through surface resonators afford precise measurement of cell mass. This then constitutes the basis for determining cell growth rate. Cell growth occurs in "recovery" channels where time is provided for cell growth, which is the key innovation in the authors' device. However, the approach suffers from a number of deficiencies whose provenance lies in how cells respond to narrow channels as well as whether cells tested require adherent-dependent growth. Firstly, cells' growth in microchannels may be fundamentally different from that experienced during cultivation in shake flasks, and thus, data obtained could not be translated to larger volume cultivation common in biotechnology experimentations. Second, stress experienced by cells during movement in microchannel may retard cell growth. Next, the authors' device could not cater to cells that require adhesion to surfaces for growth. Finally, while "recovery" channels are provided for cell growth after mass measurement, actual duration of growth phase may be shorter than time spent in "recovery" channels, and thus, this translates to underestimation of cell growth rates. Overall, an innovative solution has been found for determining cell growth rates at the single cell level. But, the approach used could introduce serious artefacts into the conceptualization of cell growth measurement. Chief amongst which is the stress experienced by cells in microchannels that could retard cell growth. While expanding the size of the channels for flowing cells may be an option, it could not resolve the above challenge, and the deficiency remains a serious one for microfluidic-based surface resonator measurement of cell growth rates.

Keywords: cell growth, distribution of growth rates, microchannels, fluid dynamics, non-adherent growth, bacteria, mammalian cells,

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A recent study in *Nature Biotechnology* ("High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays")[1] describes the use of a microfluidic based resonator for measuring the growth rates of cells via following changes in mass with time. Specifically, the resonator has been shown, through a series of experiments, to be capable of precise measurement of mass of cells by monitoring changes in the resonance frequency of an oscillator making the measurement (Figure 1).

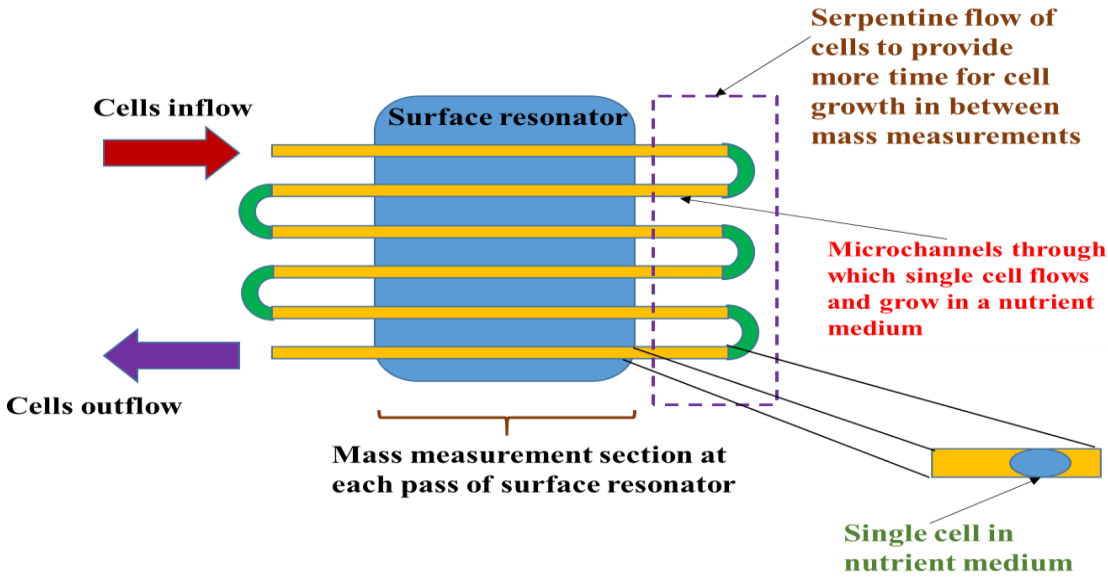

Figure 1: Schematic diagram illustrating the concept underlying the use of surface resonator in measuring growth rates of various cell types.

While the concept of measuring growth rates of cells at the single cell level through a microfluidics resonator approach allow the unprecedented delineation of individual cell growth rate, the need for individual cells to flow through narrow microchannel in between measurements to allow sufficient time for measurable growth and changes in mass to occur may perturb the measurement itself. Specifically, the measurement suffers from two potential artefacts: (i) the possible effect of constraining cells in narrow channel on cell growth rate, and (ii) the disturbances encountered in the cells as they flow through the resonator repeatedly for mass measurement after which they would have to reside in serpentine narrow channels for "growth" to occur.

Culturing cells in narrow microchannels does not mimick the natural sphere of nutrients that surrounds a cell, for example, in a shake flask. In effect, we do not know if cells could sense the dimensional constraints on their growth exerted by nearby microchannels which are of

dimension similar to the size of cells (i.e., micrometer). Hence, growth rates thus measured may not be representative of what occurs when cells are cultivated in a large volume of medium (100 mL) in a 250 mL shake flask or in a 75 cm^2 tissue culture flask. More importantly, while microbial cells typically do not require cell adhesion to surfaces for growth, mammalian cells typically need to adhere to surfaces for growth through formation of a cell adhesion layer. Flowing single cell through a narrow channel is thus, a significantly different growth paradigm from what is observed at the marcoscopic phenomenological scale.

Secondly, cells respond to changes in their environment at a variety of time scales depending on the time constant (i.e., typical amount of time required for a specific change to occur) of the process in operation. Thus, as cells flow in straight and serpertine movement through a series of microchannels, a series of fluidic disturbances are exerted on the cells, which may impact on their growth characteristics. Hence, while the authors are measuring a collection of growth rates from individual cells put through the flow channel growth experiment, a distribution of growth rates is expected since differences in individual cells' response to the fluidic stress exists. Whether the distribution would be skewed in a specific direction through unknown environmental factors' effect on cell growth in narrow microchannels is not known, but effect of growth environment on cell growth is unlikely to be random.

Imagine the journey through the microchannel based resonator from the perspective of a cell, the cell would have to pass through a repeated series of straight channels followed by loops in and out of a resonator for mass measurement, the periodic stress incurred during this fluidic movement is likely to have an effect on cell growth. Specifically, cell growth may exhibit periodic burst from heighted protein and enzyme activity as the cells "recover" in the narrow channels away from the resonator. However, this effect is not captured at both the molecular and cellular level. Obliterating this detail meant that growth rate is calculated by the change in mass over the total time that the cell spent between visits to the resonator where, in actual fact, cell growth may only occur in the time spent in narrow channel away from the resonator. More importantly, it has to be noted that time available for growth to occur may be less than the transit time in the narrow "recovery" channels.

Collectively, a microfluidic based resonator has been demonstrated to be useful for elucidating as yet unknown cell growth rate at the single cell level. However, differences in how microbial and mammalian cells approach growth with respect to the need for adherence to surfaces meant that the approach is more applicable for microbes or mammalian cells not requiring adherent growth. While the approach allows the precise measurement of mass for assessing growth performance of single cell in a flow environment, it is not representative of the

micro-environment of cells in a shake flask. More importantly, repeated transits between the resonator region and microchannels dedicated for sufficient growth to occur in between measurements meant that periodic oscillations in cellular behavior and gene expression may be present but which are not captured by the lumped parameter of growth. Specifically, stress at the resonator measurement phase may mean that cell growth occurs in the transit channel phase where cells “recover” to resume growth. Overall, the innovative approach may introduce disturbances that ultimately affect the growth rates measured. But, in science, taking the first step is far more important as it opens the windows of opportunities into the unknown as well as providing us with practical experience of the areas requiring improvements.

References

1. Cermak, N. *et al.* High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays. *Nat. Biotechnol.* **34**, 1052–1059 (2016).

Conflicts of interest

The author declares no conflicts of interest.

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