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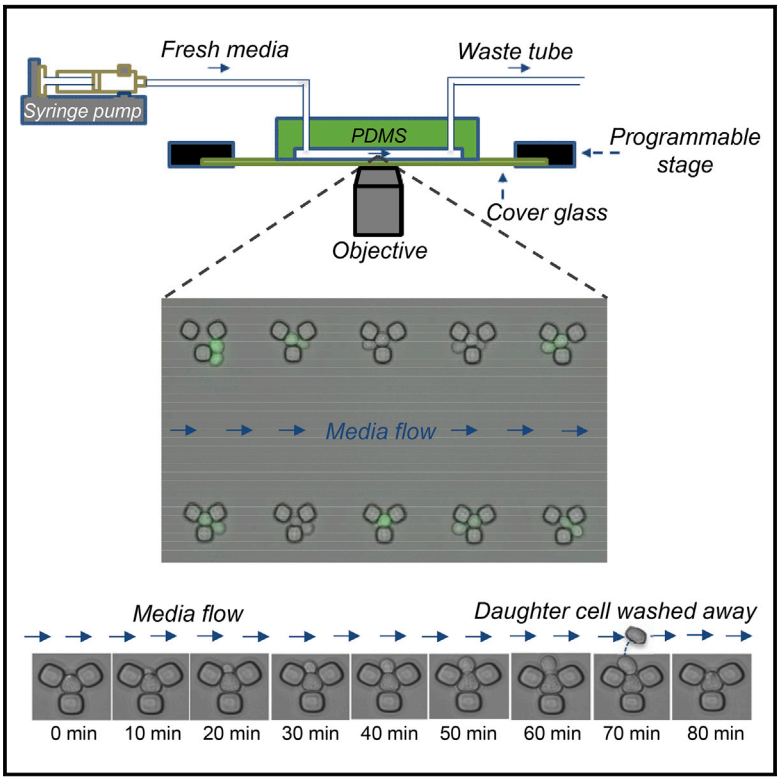
Yeast Replicator: A High-Throughput Multiplexed Microfluidics Platform for Automated Measurements of Single-Cell Aging

Graphical Abstract



Highlights

- Aging yeast cells are tracked to measure lifespan and real-time gene expression
- Single-cell lifespan is measured precisely by starting from virgin cells
- Oxidative stress levels are higher in galactose environment compared to glucose
- Aging cells commit to a single phenotypic state throughout their lifespan

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In Brief

The conventional method to measure replicative lifespan of yeast cells requires the use of micromanipulators. However, this technique has several limitations in addition to being labor intensive. Liu et al. demonstrate an automated microfluidics platform that facilitates simultaneous lifespan and gene expression measurements at the single-cell level for aging yeast cells.

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Yeast Replicator: A High-Throughput Multiplexed Microfluidics Platform for Automated Measurements of Single-Cell Aging

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SUMMARY

The yeast *Saccharomyces cerevisiae* is a model organism for replicative aging studies; however, conventional lifespan measurement platforms have several limitations. Here, we present a microfluidics platform that facilitates simultaneous lifespan and gene expression measurements of aging yeast cells. Our multiplexed high-throughput platform offers the capability to perform independent lifespan experiments using different yeast strains or growth media. Using this platform in minimal media environments containing glucose, we measured the full lifespan of individual yeast cells in wild-type and canonical gene deletion backgrounds. Compared to glucose, in galactose we observed a 16.8% decrease in replicative lifespan accompanied by an ~2-fold increase in single-cell oxidative stress levels reported by P_{SOD1}-mCherry. Using P_{GAL1}-YFP to measure the activity of the bistable galactose network, we saw that OFF and ON cells are similar in their lifespan. Our work shows that aging cells are committed to a single phenotypic state throughout their lifespan.

INTRODUCTION

Due to its short generation time and the abundance of genetic manipulation techniques, the yeast *S. cerevisiae* has been a commonly used eukaryotic model organism for aging studies (Mortimer and Johnston, 1959; Müller et al., 1980; Kaeberlein et al., 2005; Steinkraus et al., 2008; Breitenbach et al., 2012; Longo et al., 1996; Fabrizio and Longo, 2003). Being a single-cell organism, yeast allows researchers to study in vivo organismal aspects of eukaryotic aging, as numerous genetic and cell biological processes are conserved between yeast and higher eukaryotes. Two different aging models can be studied by using yeast. The first model, replicative aging (Steinkraus et al., 2008; Breitenbach et al., 2012), is a measure of the number of daughter cells a mother cell mitotically produces before it senesces. The total number of daughter cells produced determines the replicative lifespan (RLS) of the mother cell. The second model, chronological aging (Breitenbach et al., 2012; Longo et al., 1996; Fabrizio and Longo, 2003), is a measure of how long a mother cell can live in a metabolically inactive state without losing the ability to revive itself when transferred to nutrient rich media. Here, we describe an automated platform to measure RLS in real time. Our platform can also be used for chronological aging measurements, which are relatively easier to perform due to their static nature.

For several decades, the conventional method to measure yeast RLS has required the use of micromanipulators (Steinkraus et al., 2008; Breitenbach et al., 2012). Mother cells are grown and followed on solid media environments, and, to prevent crowding, each newborn daughter cell is physically separated from its mother using the micromanipulator. Typically, dozens of mother cells are processed to obtain sufficient statistics. This technique has several drawbacks. First, it is very labor intensive and requires around-the-clock mother-daughter dissection. Since a mother cell can live dozens of generations, if performed uninterrupted, a single RLS experiment can take several days. This forces researchers to refrigerate the cells overnight and continue the micromanipulation process the next day. These unavoidable temperature fluctuations would complicate the interpretation of the results, as we do not comprehensively know how growth temperature dynamics affect the aging process. Second, the micromanipulation process can physically damage the mother cells and can lower the RLS depending on the level of damage. Third, cells growing on solid media environments can have cell-to-cell differences in their exposure to the two-dimensional plate surface. This is due to the fact that the contact surface area of large and small cells would be different, leading to differences in the transportation dynamics of the nutrients into the cells.

These drawbacks have recently forced researchers to use automated microfluidic devices (Ryley and Pereira-Smith, 2006; Lee et al., 2012; Zhang et al., 2012) for measuring RLS in liquid media environments. The first such study (Ryley and Pereira-Smith, 2006) reported the use of three different designs and compared their relative efficiencies in terms of measuring yeast RLS. However, even the best-performing design identified in this study could easily trap several cells, instead of just the