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Minireview

Review of methods to probe single cell metabolism and bioenergetics

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ABSTRACT

Single cell investigations have enabled unexpected discoveries, such as the existence of biological noise and phenotypic switching in infection, metabolism and treatment. Herein, we review methods that enable such single cell investigations specific to metabolism and bioenergetics. Firstly, we discuss how to isolate and immobilize individuals from a cell suspension, including both permanent and reversible approaches. We also highlight specific advances in microbiology for its implications in metabolic engineering. Methods for probing single cell physiology and metabolism are subsequently reviewed. The primary focus therein is on dynamic and high-content profiling strategies based on label-free and fluorescence microspectroscopy and microscopy. Non-dynamic approaches, such as mass spectrometry and nuclear magnetic resonance, are also briefly discussed.

1. Introduction

Understanding metabolism and energy flow through cells has recently generated considerable interest. This is due to implications in metabolic engineering and the use of microbes as factories for the production of chemical compounds. Exploratory or optimization investigations to this end commonly take place within the widely available flasks and Petri dishes, as well as dedicated bioreactors (Fig. 1a and b). In these, many cells ($>10^{11}$) are simultaneously stimulated and their output is collectively analyzed.

In population level studies, however, the performance of individuals is masked and stimuli gradients across the population are enforced due to the geometric boundary conditions of the growth microenvironment. Single cell methods need to be implemented to address these, as recently evidenced by the wide success of single cell genomic and proteomic approaches. Herein, we review methods for studying single cell physiology – specifically metabolism and bioenergetics – where cells are now treated as isolated biochemical factories, thus enabling the precise measurement of (a) their metabolic profile, (b) culture heterogeneity, as well as (c) the effect of the microenvironment.

Single-cell experiments offer the potential in gaining a unique insight in cell metabolism. It is however, important to note that care has to be taken when designing single cell experiments and interpreting results. In addition to the need for statistical significance, the differences between experimental conditions at the population-level and the microvolume that contains an individual cell need to be considered. Such differences include population-level characteristics that are challenged by isolating an individual, such as cell-to-cell communication (e.g. via quorum sensing), spatiotemporal gradients of stimuli and the co-existence of cells in various growth stages.

The review is divided in two parts. In the first, we discuss methods for biomass manipulation down to the single cell level. This is a critical step, as the volume of a typical microbial cell (bacteria or yeast) is approximately 12 orders of magnitude smaller than common reactor volumes (~L). This is highlighted in Fig. 1a–c through the scale comparison between typical industrial and laboratory scale reactors with that of an individual *Yarrowia lipolytica* yeast cell. Thus, the study of single cells necessitates appropriate sampling and manipulation to address this considerable volume mismatch. This can be achieved *via* multiple strategies, both stochastic (*e.g.* serial dilutions) and deterministic (*e.g.* micromanipulators).

In the second part of the review, we discuss methods for probing the metabolism and bioenergetics of single cells. Areas such as single cell mass-spectrometry, bioimaging, optical sensing and spectroscopy are covered. The applications of such methods, such as strain selection, profiling intracellular metabolites, and dynamic metabolic mapping (*e.g.* respiration monitoring), will also be discussed for each individual technique.

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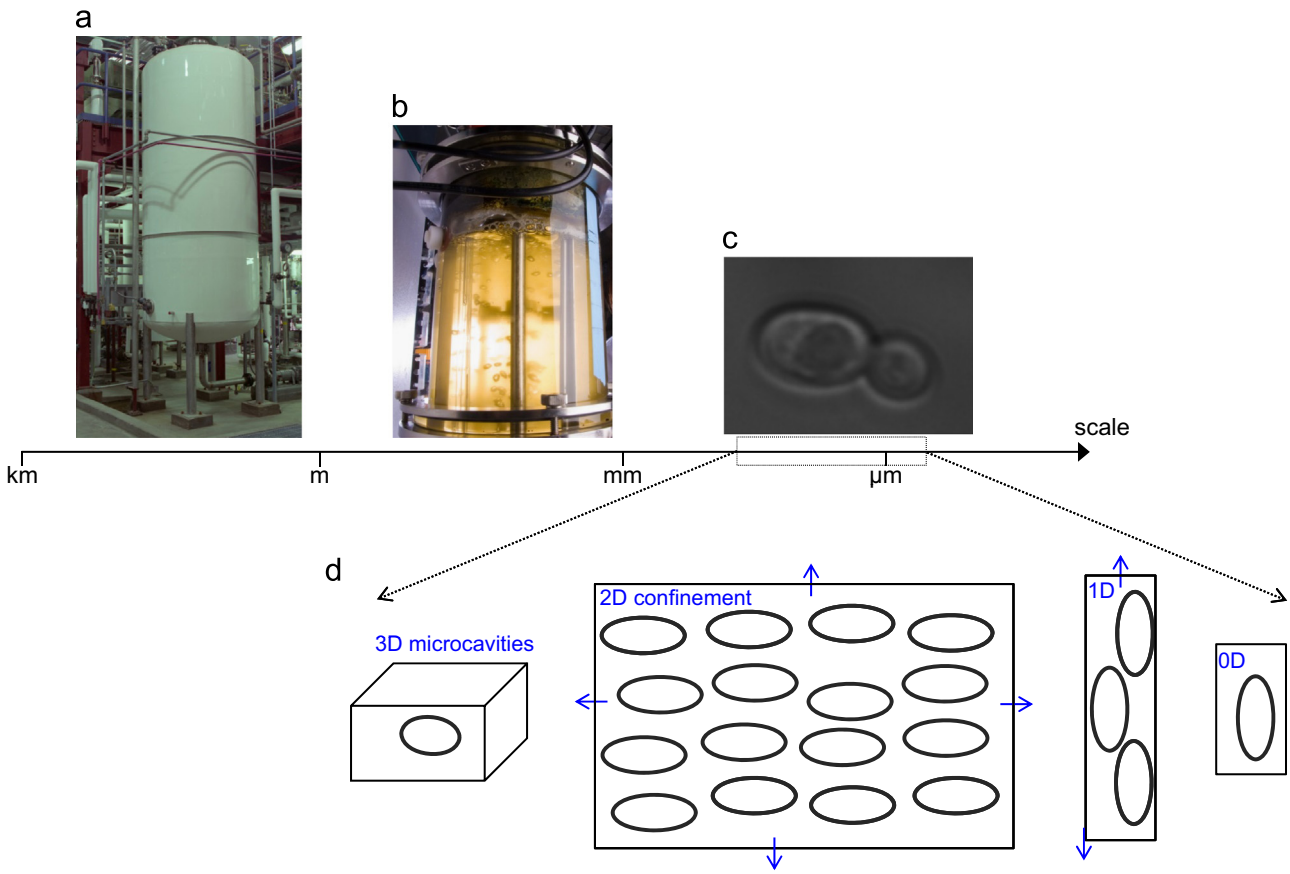

Fig. 1. *From many down to the single cell*: (a) An industrial scale fermenter with an approximate height of a few meters (credit U.S. Department of Energy). (b) A bioreactor growing algae; the vertical dimension of the instrument is a few cm containing approximately 10^{12} cells (credit U.S. Department of Energy). (c) A budding *Yarrowia lipolytica* yeast, with the daughter cell exhibiting an approximate 2 μm diameter. (c) Schematic representations of the 2D, 1D and 0D confinement types discussed in this review.

2. Single cell manipulation

The sampling and manipulation of cells down to the individual has been of substantial interest since the very beginning of Life Sciences. One of the first recorded single cell study involved the use of a micropipette to place a single *Erwinia amylovora* cell on an apple blossom and study its infectivity (Hildebrand, 1937). This technique – with minor modifications – served with great success early physiology, (Mortimer and Johnston, 1959) genetics (Zelle, 1951) and microbiology (Hildebrand, 1950) studies. Since then, a significantly wider variety of methods has emerged. These are primarily based on modern micro- and nanotechnologies involving advanced fabrication, materials and chemical functionalization methods.

Herein, our objective is to highlight the most recent developments in single cell manipulation, as well as pioneering ones; it is also worth noting that excellent reviews exist on similar topics (Andersson and van den Berg, 2003; Brehm-Stecher and Johnson, 2004; Nilsson et al., 2009; Schmid et al., 2010; Lecault et al., 2012; Zenobi, 2013; Huang et al., 2014; Grünberger et al., 2014; Avesar et al., 2014; Mu et al., 2013; Bennett and Hasty, 2009). First, *flow-through* methods will be discussed, namely: methods in which single cells flow continuously in an ordered manner during their analysis. This section will be followed by *confinement* techniques that enable cell isolation and confinement in zero-, one, or two-dimensions (Fig. 1d) (Grünberger et al., 2014). Such confinement techniques can be further divided into permanent and reversible or dynamic ones and can be based on both chemical and physical approaches. The section will conclude with a comparison between these approaches.

A critical aspect of both *flow-through* and *confinement* strategies is the enabling fabrication method, which in the majority of examples is based on Soft Lithography. The latter will not be discussed in detail in the present review and the potentially interested reader is referred to recent excellent recent reviews on the topic (Xia and Whitesides, 1998; Quake and Scherer, 2000; Weibel et al., 2007).

2.1. Flow-through methods

Flow cytometry and droplet microfluidics are the two most common methods of flow-through analysis. While both are high-throughput techniques (Hong et al., 2009), their difference is that the droplet encapsulated cells experience a restricted and personal microenvironment, while in flow cytometry cells experience similar nutrient and stimuli initial concentrations. These methods are well established and they have recently enabled immense strides in single cell phenotypic analysis, namely the identification and analysis of metabolically distinct individuals from an isogenic population using both droplet microfluidics (Wang et al., 2014) and flow cytometry (van Heerden et al., 2014).

2.1.1. Flow cytometry

Similar to its first ever designs (Croslandtaylor, 1953; Kamentsk et al., 1965), most modern flow cytometers contain a nozzle and a flow chamber. These, through the principle of hydrodynamic focusing, guide individual cells to flow sequentially through a microanalysis location, mostly optical (*i.e.* fluorescence or light scattering) or mass spectrometry based. In this way, high content