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Single-cell PCR of genomic DNA enabled by automated single-cell printing for cell isolation

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ABSTRACT

Single-cell analysis has developed into a key topic in cell biology with future applications in personalized medicine, tumor identification as well as tumor discovery (Editorial, 2013). Here we employ inkjet-like printing to isolate individual living single human B cells (Raji cell line) and load them directly into standard PCR tubes. Single cells are optically detected in the nozzle of the microfluidic piezoelectric dispenser chip to ensure printing of droplets with single cells only. The printing process has been characterized by using microbeads (10 μm diameter) resulting in a single bead delivery in 27 out of 28 cases and relative positional precision of ±350 μm at a printing distance of 6 mm between nozzle and tube lid. Process-integrated optical imaging enabled to identify the printing failure as void droplet and to exclude it from downstream processing. PCR of truly single-cell DNA was performed without pre-amplification directly from single Raji cells with 33% success rate ($N=197$) and C_q values of 36.3 ± 2.5. Additionally single cell whole genome amplification (WGA) was employed to pre-amplify the single-cell DNA by a factor of >1000. This facilitated subsequent PCR for the same gene yielding a success rate of 64% ($N=33$) which will allow more sophisticated downstream analysis like sequencing, electrophoresis or multiplexing.

1. Introduction

Even recently divided cells in culture vary in their genotype and phenotype (Spencer and Sorger, 2011; Spencer et al., 2009) and many diseases are initiated by cellular abnormalities in a small minority of cells (Asano et al., 1996; Kumaresan et al., 2008). Therefore the isolation of single cells from a heterogeneous cell population to enable single-cell assays is an upcoming task in cell research, diagnostics and therapeutics for e.g. disease prognosis and treatment evaluation (Spratlin et al., 2009). Instruments and technologies enabling isolation of individual single cells are also required for the generation of fully human or monoclonal antibodies (Tiller et al., 2008) which are an essential tool for the understanding of disease mechanisms and to improve future therapies (Karimiani and Day, 2013) especially in terms of cancer research (Bendall and Nolan, 2012; Voronin et al., 2014) and early cancer diagnosis. Furthermore single-cell isolation is applied in research concerning autoimmune diseases e.g. rheumatoid arthritis (Amara et al., 2013) or virus induced diseases like HIV or influenza (Corti and Lanzavecchia, 2013; Corti et al., 2013). Additionally single-cell related research depicts a major role in paving the way for personalized medicine which is preferably studied at the level of the patients cellome (Diks and Peppelenbosch, 2004; Irish et al., 2004). To enable all these applications, the separation of single living cells from a suspension is prerequisite. The C1™ Single Cell Autoprep system (Fluidigm Corporation, USA) is a commercially available lab-on-a-chip system enabling cell

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separation from suspension and single-cell PCR as well as whole genome amplification on a chip. Although the integration of cell separation and subsequent amplification within a chip is a great advantage for some workflows, it also has limitations. With a maximum of 96 cells the C1™ Single Cell Auto Prep IFC chip is limited in parallelization as well as cell size variation. Due to necessary pipetting steps, single-cell samples are transferred to another analysis or read-out instrument after (pre)amplification. Therefore it offers no opportunity to transfer single cells directly into common thermocyclers to perform PCR or WGA. It does neither allow the selection of user defined specific cell types nor the extraction of individual single cells for subsequent downstream analysis or any analysis technique other than PCR-based assays (Thompson et al., 2014). However there is a variety of academic lab-on-a-chip approaches employing alternative single-cell analysis methods (Lecault et al., 2012; Zhang and Xing, 2010). Therefore instruments for off-chip handling of single cells offer increased flexibility with respect to the choice of further downstream analysis methods and enrich the portfolio for single-cell analysis. A defined deposition of single cells on any off-chip substrate enables integration in standard workflows and provides the ability to use conventional analysis instruments. This may be performed by conventional cell sorting techniques such as continuous flow cytometry, e.g. fluorescence-activated cell sorting (FACS). However fluorescent labeling, fluidic shear stresses, FACS process related laser radiation and electrical droplet charging might affect cell viability negatively (Shapiro et al., 2013). Furthermore minimum required sample volumes of several hundred microliters as well as low cell deposition efficiency of 60% render these technologies non-applicable for many applications (Reiter et al., 2011).

The drop-on-demand inkjet-like printing of individual single cells (Yusof et al., 2011; Gross et al., 2013) is the ideal platform technology to enable a variety of single-cell assays. It offers flexibility with respect to target reaction vessels, e.g. microwell plates and PCR tubes, reduces excess sample volume around the desired cells and allows for automated single cell handling. Here we employed drop-on-demand inkjet-like printing of single cells to isolate individual cells and to perform highly efficient single-cell PCR of genomic DNA using a standard thermocycler and standard PCR tubes. The proposed workflow is therefore readily capable of being integrated in conventional laboratory processes. In many single-cell assays, such as antibody screening, rare cell identification and especially single-cell genome or transcriptome analysis, double-cell events must be avoided. The combination of inkjet-like printing with optical, label-free single-cell detection provides confirmation of single-cell deposition and integrity.

2. Materials and methods

2.1. Single-cell printer (SCP)

The single-cell printer has been presented and studied in detail in previous works (Yusof et al., 2011; Gross et al., 2013). It is comprised of a three-axis lab robot, an inkjet-like piezoelectric picoliter dispenser and a camera with zoom optics. The dispenser chip is made from silicon and Pyrex, which allows for optically detecting cells in the chip. The droplet generation is driving by a piezoelectric stack actuator, which deflects a 260 μm thick and approximately 1 mm × 3 mm wide membrane. The nozzle orifice is microstructured to have a 40 μm × 40 μm rectangular shape. The nozzle area of the dispenser chip is imaged and an object recognition algorithm detects individual cells. The number of cells that will be ejected within a droplet is predicted by an algorithm before dispensing (Fig. 1a). Empty droplets or droplets containing multiple cells are deflected into waste by a vacuum shutter. If a single cell is detected, the shutter is turned off and the cell is printed onto a substrate, typically microwell plates.

The microfluidic dispenser chip is glued into a PMMA chip holder to form the printing cartridge. 10 μl of cell suspension is pipetted into the cartridge's reservoir. Capillary filling transports the suspension as far as the nozzle orifice and the printing can start immediately. For subsequent PCR, single cells can also be dispensed from the microfluidic dispenser chip into tubes or tube lids (Fig. 1b) at a typical throughput of 5–10 cells/min. If only a small amount of buffer is prefilled in the tube, there is a risk of

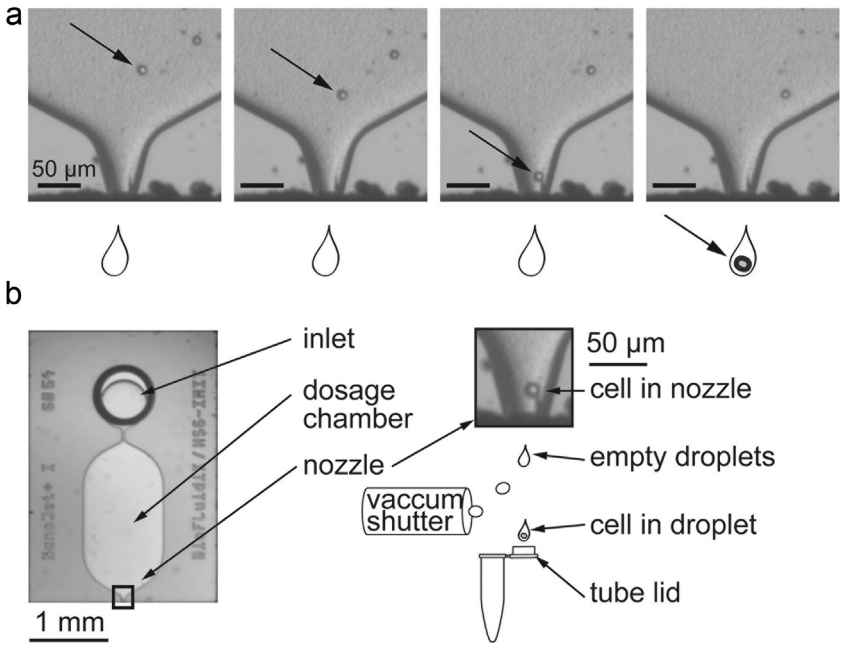

Fig. 1. (a) Image sequence of single particle dispensing. Cells or particles move closer to the nozzle orifice with each droplet dispensing. By observing the fluid volume to be dispensed with the next droplet, cell and particle count can be predicted. (b) The micrograph of the dispenser chip and close up of the nozzle region show how a single Raji cell is visually detected in the nozzle. The vacuum shutter and the droplet dispensing into a PCR tube lid is shown schematically.